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(54) **Cosmetic compositions for conditioning the hair or the skin**

(57) The cosmetic composition consists of two separate liquid phases, the first phase being an oily phase and the second phase being an aqueous phase containing at least one cationic polymer in the dissolved state.

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SPECIFICATION

Cosmetic compositions for conditioning the hair or the skin, and their application

The present invention relates to cosmetic compositions for conditioning the hair or the skin, and their application.

Oils and mixtures of oils have always been used for conditioning the hair, in particular dry or sensitised hair, that is to say hair which has been degraded by exposure to inclement environments (sun, sea water and the like) or also by treatments such as perming, colouring and bleaching.

Similarly, oily cosmetic compositions have been used for conditioning the skin, that is to say for making dry, wrinkled or rough skin supple and soft to the touch.

When applied to the hair, after rinsing and/or shampooing, oils, despite their ease of application and impregnation, impart the desired properties, that is to say an appreciable softness to the touch, a glossy appearance and a protective effect against the environment, only to a small extent. Likewise, the use of a purely oily composition for the skin is not entirely satisfactory, in particular because of the difficulties of uniform spreading, the resulting greasy appearance and the "greasy" sensation which is unpleasant to the touch.

It is for this reason that the use of oil-based products has for some time been rather neglected in favour of other, more effective products for conditioning and protecting sensitised hair and the skin. These new products, which are used in modern cosmetic compositions, are especially polymers of the polyamine, polyaminoamide or poly - (quaternary ammonium) type, the amine or ammonium groups forming part of the polymer chain or being joined to the latter. Polymers of this type, and their use in cosmetic compositions, have been described in numerous publications, and cosmetology specialists have grouped all these polymers together in a class which is generally designated by the expression "cationic polymers". Although these cationic polymers can have varied effects, specialists have recognised that they all have the common property, albeit to various extends, of attaching themselves to the skin and the hair and of making the hair easier to comb out when wet.

However, attempts to incorporate cationic polymers into oils have not made it possible to produce effective cosmetic compositions. In certain cases, it has been impossible to incorporate the cationic polymers into the oils because the said polymers could not be isolated in the dry state and only existed in aqueous solution, or the polymers were not soluble in the oils. In other cases, it was possible to incorporate the polymer into the oils, but the effectiveness of the polymers was then very low because their ability to attach to the skin or the hair was inhibited in the oily medium.

A further possibility for combining the effects of oils and cationic polymers was first to apply an aqueous solution of cationic polymer and then to apply an oil. However, these attempts also failed because either the amount of water (aqueous solution of cationic polymer) is too small and it is not possible to distribute it uniformly over the whole head of hair, or the amount of water is sufficient for an efficient distribution over the hair, but the oil is then distributed poorly over the hair when wet. Likewise, the oil is distributed poorly over the skin when wet.

It has now been discovered that it is possible to combine the cosmetic effects of oils and cationic polymers by virtue of a particular, non-detergent and non-foaming composition, which forms the subject of the present invention.

The present invention provides a cosmetic composition for conditioning the skin or the hair, characterised in that it consists of two separate liquid phases, the first phase being an oily phase and the second phase being an aqueous phase in which at least one cationic polymer is dissolved, and in that the said composition is free of detergents and foaming agents.

The oils which can be used in the compositions of the present invention are in general the oils which can be used in cosmetic products for the skin or for the hair.

These are especially vegetable oils, such as sweet-almond oil, groundnut oil, wheatgerm oil, linseed oil, jojoba oil, apricot stone oil, walnut oil, palm oil, pistachio nut oil, sesame oil, rapeseed oil, cade oil, maize germ oil, peach stone oil, poppy seed oil, pine oil, castor oil, soya oil, avocado oil, safflower oil, coconut oil, hazelnut oil, olive oil, grapeseed oil and sunflower seed oil.

The oily phase of the compositions of the invention can also contain animals oils, such as whale oil, lard oil, horsehoof oil, tuna oil, caballine oil, otter oil, egg oil, sheep oil, seal oil, turtle oil, halibut liver oil, marmot oil, cod liver oil, neat's-foot oil and tallow oil.

The oily phase can also contain a synthetic oil preferably consisting of carbon, hydrogen and oxygen, such as the glycol ethers or esters or glycerol ethers or esters described in French Patents No. 74/09,657, No. 75/24,656 and Nos. 75/24,657 and 75/24,658.

The oily phase can also contain a mineral oil, such as vaseline oil (liquid petrolatum).

Preferably, the oily phase is free of silicone oils.

In general, it is preferred to use a vegetable oil mixed, if appropriate, with an animal oil and/or a mineral or synthetic oil as defined above.

In this preferred embodiment, the proportion of vegetable oil is generally from 25% to 100% by volume, relative to the total volume of the oily phase, any remainder consisting of one or more animal, synthetic and/or mineral oils.

As indicated above, the cationic polymers which can be used in the cosmetic products for the hair constitute a class which is well known to cosmetology specialists. Cationic polymers of this type are polycationic products described, in particular, in the following French Patents and French Patent Applications: 2,077,143, 1,492,597, 2,162,025, 2,280,361, 2,252,840, 2,368,508, 1,583,363, 2,080,759, 2,190,406, 2,320,330, 2,270,846, 76/20,261, 2,336,434, 2,413,907 and 2,189,434, and in U.S. Patents 3,227,615,

2,961,347, 2,273,780, 2,375,853, 2,388,614, 2,454,547, 3,206,462, 2,261,002, 2,271,378, 3,874,870, 4,001,432, 3,929,990, 3,966,904, 4,005,193, 4,025,617, 4,025,627, 4,025,653, 4,026,945, 3,589,978, 4,031,307 and 4,027,020.

The cationic polymers used in the composition of the invention are thus generally polymers of the polyamine, polyaminoamide or poly - (quaternary ammonium) type, the amine or ammonium group forming part of the polymer chain or being joined to the latter.

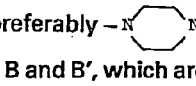
Polymers of this type which can be used according to the invention are especially:

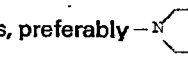
1. vinylpyrrolidone/aminoalcohol acrylate or methacrylate copolymers (quaternised or unquaternised), such as those sold under the name Gafquat by the Gaf Corp., such as "copolymer 845" and "Gafquat 734 or 755", described in greater detail, in particular, in French Patent 2,077,143;

2. cellulose ether derivatives containing quaternary ammonium groups, such as those described in French Patent 1,492,597 and especially the polymers sold under the name JR, such as JR 125, JR 400 and JR 30 M, and under the name LR, such as LR 400 and LR 30 M, by the Union Carbide Corp., and cationic cellulose derivatives, such as CELQUAT L 200 and CELQUAT L 60 and H 100 sold by National Starch;

3. cationic polysaccharides, such as those described in U.S. Patents 3,589,978 and 4,031,307, and in particular Jaguar C 13 S sold by MEYHALL and CELANESE;

4. cationic polymers chosen from:

a) polymers containing units of the formula: –A–Z–A–Z–, in which A denotes a radical containing two terminal amino groups, preferably  and Z denotes the symbol B or B'; B and B', which are identical or different, denote a divalent radical which is a straight or branched-chain alkylene radical which contains up to 7 carbon atoms in the main chain, which is unsubstituted or substituted by one or more hydroxyl groups and which can also contain chain oxygen, nitrogen or sulphur atoms and 1 to 3 aromatic and/or heterocyclic rings, the oxygen, nitrogen and sulphur atoms being present in the form of an ether or thioether, sulphoxide, sulphone, sulphonium, amine, alkylamine, alkenylamine, benzylamine, amine oxide, quaternary ammonium, amide, imide, alcohol, ester and/or urethane group; these polymers and the process for their preparation are described in French Patent 2,162,025;

b) polymers containing units of the formula: $-A-Z_1-A-Z_1-$, in which A denotes a radical containing two terminal amine groups, preferably  , and Z_1 denotes the symbol B_1 or B'_1, being the symbol B'_1 at least once; B_1 denotes a divalent radical which is a straight- or branched-chain alkylene or hydroxyalkylene radical having up to 7 carbon atoms in the main chain, and B'_1 is a divalent radical which is a straight- or branched-chain alkylene radical which has up to 7 carbon atoms in the main chain, which is the unsubstituted or substituted by one or more hydroxyl radicals and which is interrupted by one or more nitrogen atoms, the nitrogen atom being optionally substituted by an alkyl chain which has from 1 to 4, and preferably 4, carbon atoms, which is optionally interrupted by an oxygen atom and which optionally contains one or more hydroxyl groups; these polymers and the process for their preparation are described in French Patent 2,280,361; and

c) the quaternary ammonium salts and the oxidation products of the polymers of the formulae indicated above under a) and b);

5. optionally alkylated, crosslinked polyaminoamides chosen from at least one water-soluble crosslinked polymer obtained by crosslinking a polyamino - polyamide (A) prepared by the polycondensation of an acid compound with a polyamine, the acid compound being chosen from: (i) organic dicarboxylic acids, (ii) aliphatic monocarboxylic and dicarboxylic acids with an ethylenic double bond, (iii) the esters of the abovementioned acids, preferably the esters with lower alkanols having from 1 to 6 carbon atoms, and (iv) mixtures of these compounds. The polyamine is chosen from amongst bis-primary and mono- or di-secondary polyalkylene - polyamines. Up to 40 mol % of this polyamine can be replaced by a bis-primary amine, preferably ethylenediamine, or by a bis-secondary amine, preferably piperazine, and up to 20 mol % can be replaced by hexamethylenediamine. The crosslinking is carried out by means of a crosslinking agent (B) chosen from epihalogenohydrins, diepoxides, dianhydrides, unsaturated anhydrides and bis-unsaturated derivatives, and is characterised in that it is carried out using 0.025 to 0.35 mol of crosslinking agent per amine group of the polyamino - polyamide (A) and generally using 0.025 to about 0.2, and in particular 0.025 to about 0.1, mol of crosslinking agent per amine group of the polyamino - polyamide (A). These polymers and their preparation are described in greater detail in French Patent Application No. 2,252,840.

It is also possible to use, on the one hand, the polyaminoamides A, and, on the other hand, the water-soluble crosslinked polyaminoamides obtained by crosslinking a polyaminoamide A (described above) by means of a crosslinking agent chosen from:

(I) compounds chosen from (1) bis - halogenohydrins, (2) bis - azetidinium compounds, (3) bis - halogenoacyl - diamines and (4) bis - (alkyl halides);

(II) the oligomers obtained by reacting a compound (a) chosen from the (1) bis - halogenohydrins, (2) bis - azetidinium compounds, (3) bis - halogenoacyl - diamines, (4) bis - (alkyl halides), (5) epihalogenohydrins, (6) diepoxides and (7) bis - unsaturated derivatives, with a compound (b) which is a difunctional compound which is reactive towards the compound (a); and

(III) the quaternisation product of a compound chosen from the compounds (a) and the oligomers (II) and containing one or more totally or partially alkylated tertiary amine groups, with an alkylating agent (c) preferably chosen from methyl or ethyl chlorides, bromides, iodides, sulphates, mesylates and tosylates, benzyl chloride or bromide, ethylene

oxide, propylene oxide and glycidol, the crosslinking being carried out using 0.025 to 0.35 mol, in particular 0.025 to 0.2 mol and more particularly 0.025 to 0.1 mol, of crosslinking agent per amine group of the polyaminoamide.

These crosslinking agents and these polymers, and also the process for their preparation, are described in French Application No. 2,368,508;

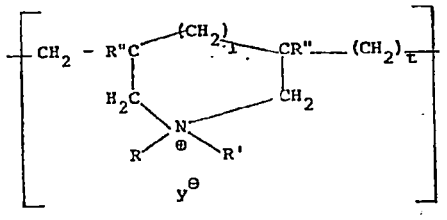
6. the water-soluble polyaminoamide derivatives resulting from the condensation of polyalkylene - polyamines with polycarboxylic acids, followed by alkylation by means of difunctional agents, such as the adipic acid/dialkylaminohydroxyl - dialylenetriamine copolymers in which the alkyl radical contains 1 to 4 carbon atoms and preferably denotes methyl, ethyl or propyl, which are described in French Patent 1,583,363.

Amongst these derivatives, there may be mentioned the adipic acid/dimethylaminohydroxypropyl - diethylene - triamine copolymers sold under the name Cartaretine F, F_4 or F_8 by SANDOZ;

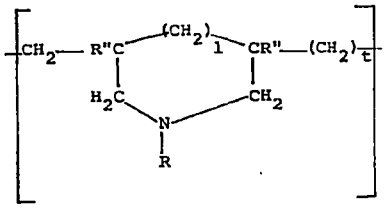
7. the polymers obtained by reacting a polyalkylene - polyamine containing two primary amine groups and at least one secondary amine group, with a dicarboxylic acid chosen from diglycolic acid and saturated aliphatic dicarboxylc acids having 3 to 8 carbon atoms, the molar ratio of the polyalkylene - polyamine to the dicarboxylic acid being 0.8:1 to 1.4:1, and the resulting polyamide being reacted with epichlorohydrin in a molar ratio of epichlorohydrin to the secondary amine groups of the polyamide of 0.5:1 to 1.8:1; these polymers are mentioned in U.S. Patents 3,227,615 and 2,961,347.

The polymers of this type are especially that sold under the name HERCOSETT 57 by Hercules Incorporated, which has a viscosity at 25°C of 30 cps in 10% strength aqueous solution, and that sold under the name PD 170 or DELSETTE 101 by Hercules, which is an adipic acid/epoxypropyl - diethylenetriamine copolymer.

8. water-soluble cyclic polymers having a molecular weight of 20,000 to 3,000,000, such as the homopolymers containing, as the main constituent of the chain, units corresponding to the formula (II) or (II'):



II



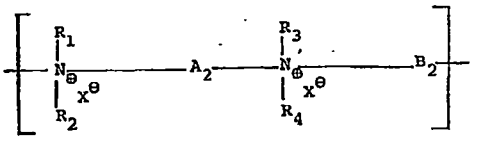
II'

in which l and t are equal to 0 or 1, t = 1 if l = 0 and 5 = 0 if l = 1, R" denotes hydrogen or methyl, R and R' independently if one another denote an alkyl group having from 1 to 22 carbon atoms, a hydroxyalkyl group in which the alkyl group preferably has 1 to 5 carbon atoms, or a lower amidoalkyl group, or R and R' denote, together with the nitrogen atom to which they are attached, hetereocyclic groups such as piperidinyl or morpholinyl, and $y^{\ominus}$ is an anion, such as bromide, chloride, acetate, borate, citrate, tartrate, bisulphate, bisulphite, sulphate or phosphate, and also the copolymers containing units of the formula II or II' and, preferably, units derived from acrylamide or from diacetone - acrylamide.

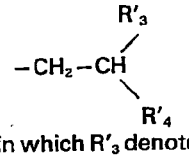
Amongst the quaternary ammonium polymers of the type defined above, there may be mentioned the dimethyl - diallylammonium chloride homopolymer sold under the name MERQUAT 100, which has a molecular weight of less than 100,000, and the dimethyldiallylammonium chloride/acrylamide copolymer having a molecular weight of more than 500,000, which is sold under the name MERQUAT 550 by MERCK.

These polymers are described in French Patent 2,080,759 and its Certificate of Addition No. 2,190,406.

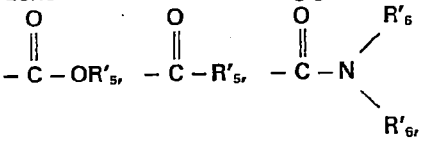
9. water-soluble poly - (quaternary ammonium) compounds containing repeat units of the formula:

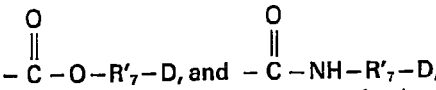


in which R_1 and R_2, and R_3 and R_4, which are identical or different, represent lower aliphatic or hydroxyaliphatic radicals having a maximum of 4 carbon atoms, it being possible however, for one of them to represent an allphatic radical containing from 5 to 16 carbon atoms, an alicyclic radical or an arylaliphatic radical, provided that, in this case, the sum of the numbers of carbon atoms in the groups A_2 and B_2 is equal to at most 12, or alternatively R_1, R_2, R_3 and R_4 together or separately form, with the nitrogen atoms to which they are attached, heterocyclic rings optionally containing a second hetero-atom other than nitrogen, or alternatively R_1, R_2, R_3 and R_4 represent a group



in which R'_3 denotes hydrogen or lower alkyl and R'_4 denotes one of the following groups: –CN,

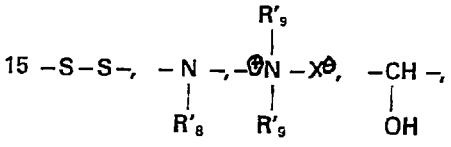


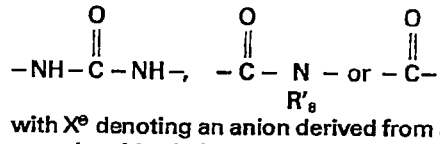


R'_5 denoting lower alkyl, R'_6 denoting hydrogen or

lower alkyl, R'_7 denoting alkylene and D denoting a quaternary ammonium group, A_2 and B_2 represent divalent groups, for example polymethylene groups containing from 2 to 20 carbon atoms, which can be linear or branched and saturated or unsaturated and which can contain, inserted in the main chain, one or more aromatic rings, such as the group

or one or more groups $-(CH_2)_n-Y_1-(CH_2)_n-$, Y_1 denoting O, S, SO, SO_2,



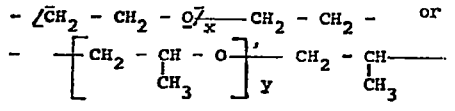


with $X^\ominus$ denoting an anion derived from a mineral or organic acid, n being 2 or 3, R'_8 denoting hydrogen or lower alkyl and R'_9 denoting lower alkyl, or alternatively A_2 and R_1 and R_3 form a piperazine ring with the two nitrogen atoms to which they are attached; moreover, if A denotes a linear or branched, saturated or unsaturated, aliphatic or hydroxyaliphatic e.g. alkylene or hydroxyalkylene radical, B can also denote a group:

$-(CH_2)_n-CO-D-OC-(CH_2)_n-$,

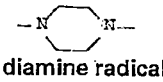
in which D denotes:

a) a glycol radical of the formula $-O-Z-O-$, in which Z denotes a linear or branched hydrocarbon radical or a group corresponding to the formulae:



in which x and y denote an integer from 1 to 4, representing a definite and unique degree of polymerisation or any number from 1 to 4, representing an average degree of polymerisation (in the case of a mixture);

b) a bis - secondary diamine radical, such as a piperazine derivative of the formula:



c) a bis - primary diamine radical of the formula:

$-NH-Y-NH-$

in which Y denotes a linear or branched hydrocarbon radical or the divalent radical $-CH_2-CH_2-S-S-CH_2-CH_2-$; or

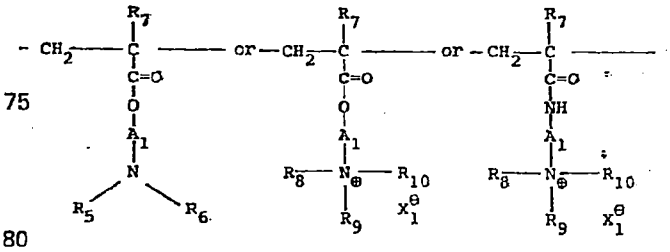
d) a ureylene group of the formula $-NH-CO-NH-$; and $X^\ominus$ is an anion, such as chloride or bromide.

These polymers have a molecular weight which is generally 1,000 to 100,000.

Polymers of this type are described, in particular, in French Patents 2,320,330 and 2,270,846, French Applications 76/20,261 and 2,336,434 and U.S. Patents 2,273,780, 2,375,853, 2,388,614, 2,454,547, 3,206,462, 2,261,002 and 2,271,378.

Other polymers of this type are described in U.S. Patents 3,874,870, 4,001,432, 3,929,990, 3,966,904, 4,005,193, 4,025,617, 4,025,627, 4,025,653, 4,026,945 and 4,027,020.

10. homopolymers or copolymers derived from acrylic or methacrylic acid and containing the unit:



in which R_7 is H or CH_3, A_1 is a linear or branched alkyl group having 1 to 6 carbon atoms or a hydroxyalkyl group having 1 to 4 carbon atoms, R_8, R_9 and R_{10}, which are identical or different, represent an alkyl group having 1 to 18 carbon atoms or a benzyl group, R_5 and R_6 represent H or alkyl having 1 to 6 carbon atoms, and X_1 denotes halogen, such as chlorine or bromine, or methosulphate.

The comonomer or comonomers which can be used are typically: acrylamide, methacrylamide, diacetone - acrylamide, acrylamide and methacrylamide substituted on the nitrogen by lower alkyl, alkyl esters of acrylic and methacrylic acids, vinylpyrrolidone and vinyl esters.

The following may be mentioned by way of example:

the acrylamide/beta - methacryloyloxyethyltrimethylammonium methodsulphate copolymer sold under the names Reten 205, 210, 220 and 240 by Hercules,

the ethyl methacrylate/oleyl methacrylate/beta-methacryloyloxyethyl - diethyl - methylammonium methosulphate copolymers listed under the name Quaternium 38 in the Cosmetic Ingredient Dictionary,

the ethyl methacrylate/abietyl methacrylate/beta-methacryloyloxyethyl - diethyl - methylammonium methosulphate copolymer listed under the name Quaternium 37 in the Cosmetic Ingredient Dictionary,

the beta - methacryloyloxyethyl - trimethylammonium bromide polymer listed under the name Quaternium 49 in the Cosmetic Ingredient Dictionary,

the beta - methacryloyloxyethyl - trimethylammonium methosulphate/beta - methacryloyloxyethyl - stearyldimethylammonium methosulphate copolymer listed under the name Quaternium 42 in the Cosmetic Ingredient Dictionary,

the aminoethylacrylate phosphate/acrylate copolymer sold under the name Catrex by National Starch, and

the graft and crosslinked cationic copolymers having a molecular weight of 10,000 to 1,000,000 and preferably of 15,000 to 500,000, which result from the copolymerisation of:

a) at least one cosmetic monomer,

b) dimethylaminoethyl methacrylate,

c) polyethylene glycol and

d) a polyunsaturated crosslinking agent,

these copolymers being described in French Patent 2,189,434.

The crosslinking agent is typically: ethylene glycol dimethacrylate, a diallyl phthalate, divinylbenzene, tetraallyloxyethane or a polyallyl sucrose.

The cosmetic monomer can be of a very varied type, for example a vinyl ester of an acid having from 2 to 18 carbon atoms, an allyl or methallyl ester of an acid having from 2 to 18 carbon atoms, an acrylate or methacrylate of a saturated alcohol having from 1 to 18 carbon atoms, an alkyl vinyl ether in which the alkyl radical has from 2 to 18 carbon atoms, an olefine having from 4 to 18 carbon atoms, a vinyl heterocyclic derivative, a dialkyl or N,N - dialkylaminoalkyl maleate in which the alkyl radicals have from 1 to 3 carbon atoms, or an anhydride of an unsaturated acid.

Other cationic polymers which can be used are, for example, polyalkyleneimines, in particular polyethyleneimines, polymers containing vinylpyridine units or vinylpyridinium units in the chain, condensates of polyamines and epichlorohydrin, poly - (quaternary ureylene) compounds and chitin derivatives.

In the compositions of the invention, the proportion of the aqueous phase is preferably from 2 to 50% by volume, relative to the total volume of the composition.

The cationic polymer which is dissolved in the aqueous phase is suitably present in the composition of the invention in an amount of 0.2 to 50% by weight, relative to the total weight of the composition.

Apart from the cationic polymer, the aqueous phase can contain, in the dissolved state, especially, preservatives, thickeners, colorants and/or water-soluble solvents, such as alcohols (in particular ethanol). If they are present, these solvents suitably represent a maximum of 50% by volume, relative to the total volume of the aqueous phase.

The oily phase can contain, especially, antioxidants, colorants and/or perfumes, in the dissolved state. The compositions of the present invention are usually free of detergents and foaming agents. More generally, they are free of anionic and amphoteric surface-active agents; most frequently, the compositions of the present application are free of any surface-active agent. However, in certain cases, they can contain a small amount (less than 3% by weight) of a cationic surface-active agent present as a cosmetic agent. In fact, it is known that cationic surface-active agents have a conditioning effect when deposited onto the hair or the skin.

This invention also relates to the application of the two-phase composition described above, that is to say its use for treating and conditioning the hair, especially dry or sensitised hair, and for treating and conditioning the skin, in particular dry, wrinkled or rough skin or skin lacking in suppleness.

In the case of hair conditioning, the composition should be shaken in order to obtain a dispersion of the aqueous phase in the oily phase; typically from 5 to 40 cm^3 of the composition shaken in this way is applied to the head of hair and the composition is left to act for at least five minutes and the hair is then rinsed. If desired, the hair can then be washed with a shampoo, preferably with an anionic shampoo.

Preferably, the composition is left to act for at least five minutes. It is of course possible to leave the composition to act for several hours.

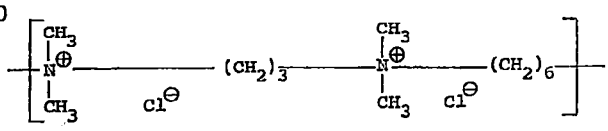
In general, this hair conditioning can be effected before or after a shampooing, colouring, bleaching, perming or straightening operation.

In the case of skin conditioning, the composition should again be shaken in order to obtain a dispersion of the aqueous phase in the oily phase, and the composition shaken in this way spread over the skin, the latter being massaged if desired. Any excess composition can be removed with an absorbent wad (of fabric, cottonwool or paper), but, in contrast to the case of the hair, the composition need not be rinsed off. Preferably, the composition is applied to skin which has been cleaned beforehand.

The following Examples further illustrate the present invention.

In these Examples, the meanings of the symbols denoting the cationic polymers are as follows:

Polymer P_1: polymer consisting of units of the formula:



which can be prepared as described in French Patents 2,270,846 and 2,333,012.

JR 400: hydroxyethylcellulose/epichlorohydrin polymer quaternised with trimethylamine, having a viscosity of 400 cps and sold by Union Carbide.

Merquat 100: dimethyldiallylammonium chloride homopolymer having a molecular weight of less than 100,000 and sold by Merck & Co.

CARTARETINE F.4: adipic acid/dimethylaminohydroxypropyl - diethylenetriamine copolymer sold by Sandoz.

Polymer P_2: polycondensate of equimolecular amounts of adipic acid and diethylenetriamine, crosslinked with epichlorohydrin (11 mols of epichlorohydrin per 100 amino groups) and described in French Patent 2,252,840.

Example 1: The oily and aqueous phases having the following compositions are prepared:

Oily phase
maize oil: 20 cc
antioxidant q.s *
perfume q.s.
colorant q.s.
sunflower seed oil q.s.p.: 100 cc

*q.s. means: sufficient quantity.

Aqueous phase
polymer P_1: 5 g (a.i. *)
preservative q.s
colorant q.s
water q.s.p: 100 cc

* a.i. means: active ingredient.

The two-phase composition is obtained by bringing together 2 cm^3 of the aqueous phase and 18 cm^3 of the oily phase.

After shaking, the composition is applied to dry or sensitised hair. After an interval of 5 minutes to 4

hours, but preferably after 15 minutes, the hair is rinsed and then shampooed.

The hair is easy to comb out when wet. The hair is soft, bulky and glossy when dry.

Comparable results are obtained by using the following compositions:

Example 2: 16 cc of oily phase and 4 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
avocado oil: 10 cc
antioxidant q.w
perfume q.s
colorant q.s
rapeseed oil q.s.p: 100 cc
Aqueous phase
polymer P_1: 1.5 g (a.i.)
JR 400: 1 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 3: 18 cc of oily phase and 2 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
castor oil: 5 cc
antioxidant q.s
perfume q.s
colorant q.s
soya oil q.s.p: 100 cc
Aqueous phase
Merquat 100: 3 g (a.i.)
Cartaretine F_4: 5 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 4: 16 cc of oily phase and 4 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
identical to that of Example 3
Aqueous phase
polymer P_1: 1.5 g (a.i.)
polymer P_2: 3.5 g (a.i.)
ethyl alcohol q.s.p: 50° strength
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 5: 16 cc of oily phase and 4 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
linseed oil: 1 cc
antioxidant q.s
perfume q.s
colorant q.s
sweet-almond oil q.s.p: 100 cc
Aqueous phase
polymer P_1: 1.5 g (a.i.)
polymer P_2: 3.5 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 6: 10 cc of oily phase and 10 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
jojoba oil: 20 cc
antioxidant q.s
perfume q.s
colorant q.s
sesame oil q.s.p: 100 cc
Aqueous phase
identical to that of Example 3

Example 7: 10 cc of oily phase and 10 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
peach stone oil: 10 cc
vaseline oil: 30 cc
preservative q.s
perfume q.s
colorant q.s
olive oil q.s.p: 100 cc
Aqueous phase
identical to that of Example 1

Example 8: 18 cc of oily phase and 2 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
identical to that of Example 7
Aqueous phase
Merquat 100: 3 g (a.i.)
Cartaretine F_4: 5 g (a.i.)
ethyl alcohol q.s.p: 25° strength
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 9: 16 cc of oily phase and 4 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
horse grease: 25 cc
antioxidant q.s
perfume q.s
colorant q.s
wheatgerm oil q.s.p: 100 cc
Aqueous phase
identical to that of Example 5

Example 10: 19.5 cc of oily phase and 0.5 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
identical to that of Example 2
Aqueous phase
polymer P_1: 60 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 11: 10 cc of oily phase and 10 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
identical to that of Example 3
Aqueous phase
Merquat 100: 0.3 g (a.i.)
Cartaretine F_4: 0.3 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

Example 12: 16 cc of oily phase and 4 cc of aqueous phase are mixed, the phases having the following compositions:

Oily phase
antioxidant q.s
perfume q.s
colorant q.s
jojoba oil q.s.p 100 cc

Aqueous phase
Merquat 100: 2 g (a.i.)
preservative q.s
colorant q.s
water q.s.p: 100 cc

After shaking, the composition is applied to the skin. It imparts suppleness and softness thereto. Similar results are obtained by applying the composition of Example 2 to the skin.

CLAIMS

1. A composition suitable for application to the skin or the hair, which comprises two separate liquid phases, the first phase being an oily phase and the second phase being an aqueous phase having dissolved therein, at least one cationic polymer.

2. A composition according to claim 1, in which the oily phase contains from 25% to 100% by volume of vegetable oil, relative to the total volume of the oily phase, any remainder consisting of one or more animal, synthetic and/or mineral oils.

3. A composition according to claim 1 or 2, in which the oily phase also contains at least one dissolved antioxidant, colorant or perfume.

4. A composition according to any one of the preceding claims, in which the oily phase is free of silicones.

5. A composition according to any one of the preceding claims, in which the cationic polymer is a polymer of the polyamine, polyaminoamide or poly-(quaternary ammonium) type, the amine or ammonium groups forming part of the polymer chain or being attached to the latter.

6. A composition according to any one of the preceding claims, in which the proportion of the aqueous phase is from 2 to 50% by volume, relative to the total volume of the composition.

7. A composition according to any one of the preceding claims, in which the cationic polymer is present in the composition in an amount of 0.2 to 50% by weight, relative to the total weight of the composition.

8. A composition according to any one of the preceding claims, in which the aqueous phase also contains at least one of a preservative, a thickener, a colorant and a water-soluble solvent.

9. A composition according to claim 7, in which the said water-soluble solvent is an alcohol.

10. A composition according to any one of the preceding claims which is free of detergent.

11. A composition according to claim 1 substantially as described in any one of the Examples.

12. A method of conditioning the hair or the skin which comprises applying thereto a composition as claimed in any one of the preceding claims.

13. A method according to claim 12, in which the composition is shaken in order to obtain a dispersion of the aqueous phase in the oily phase, from 5 to 40 cm^3 of the shaken composition are applied to the head of hair, the composition is left to act for at least 5 minutes and the hair is then rinsed.

14. A method according to claim 13, in which the hair is then washed with a shampoo.

15. A method according to claim 12, in which the composition is shaken in order to obtain a dispersion of the aqueous phase in the oily phase, the shaken composition is spread over the skin, the latter optionally being massaged.

16. A method according to claim 12 substantially as hereinbefore described.

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File: 1985-us-patent-4525342.pdf

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Donald J. Quigg

Acting Commissioner of Patents and Trademarks

Melvinia Gary

Attest

United States Patent [19]

Weiss et al.

[11] **Patent Number: 4,525,342**

[45] **Date of Patent: Jun. 25, 1985**

[54] **DENTAL AND ORAL PREPARATION**

[76] Inventors: **Ervin Weiss**, 18 Kley St., Tel Aviv; **Melvin Rosenberg**, 12 Yeda-am St., Ramat-gan; **Herbert Judas**, 5 Habrosh St., Raanana, all of Israel

[21] Appl. No.: **582,065**

[22] Filed: **Feb. 21, 1984**

[30] **Foreign Application Priority Data**

Mar. 3, 1983 [IL] Israel 68027

[51] **Int. Cl.**[3] **A61J 7/00**; A61J 7/16

[52] **U.S. Cl.** **424/49**; 222/94; 222/129.4; 222/192

[58] **Field of Search** 424/49-58; 222/94, 129.4, 192, 478, 565, 566

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Primary Examiner—Shep K. Rose

Attorney, Agent, or Firm—Bucknam and Archer

[57] **ABSTRACT**

There are provided compositions for dental and oral hygiene which desorb microorganisms and which adsorb odorous mercapto-compounds which cause halitosis. The compositions comprise as active ingredient hydrophobic materials selected from vegetable oils, certain mineral oils and hydrocarbons.

5 Claims, 2 Drawing Figures

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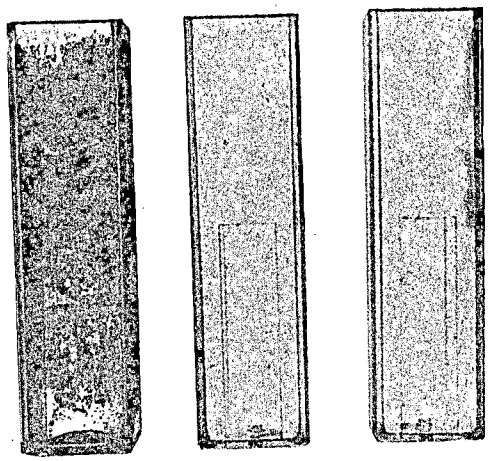

FIG.1

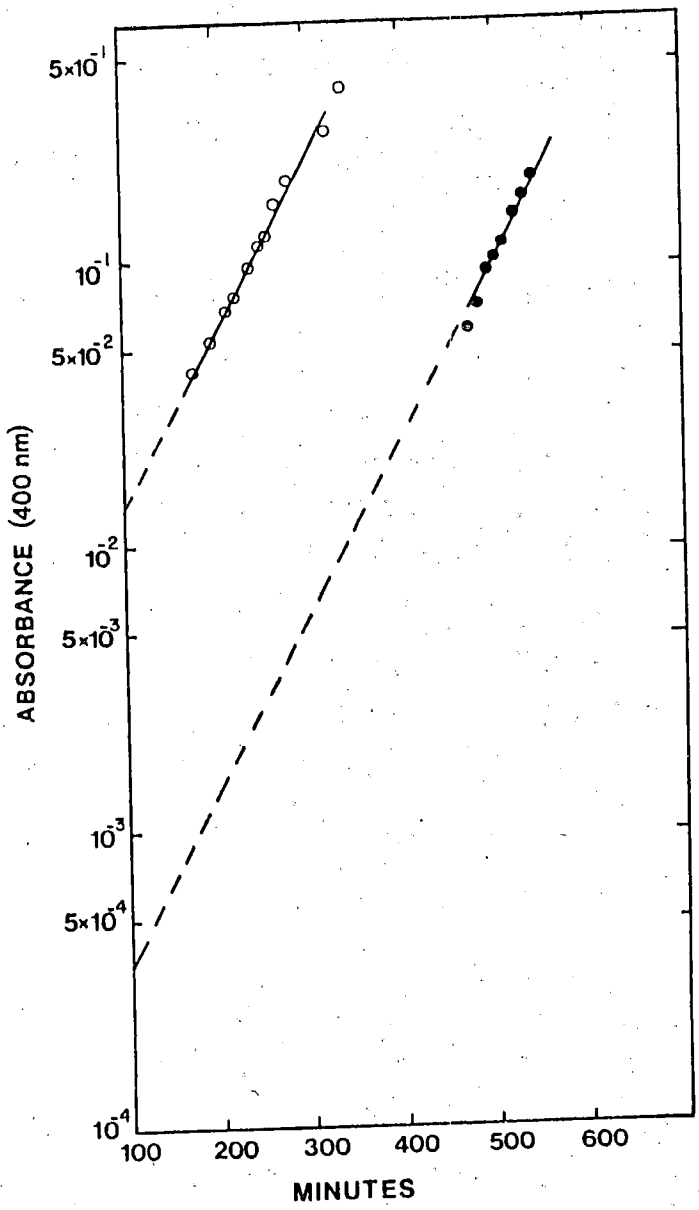
ABSORBANCE (400 nm)
5×10⁻¹
10⁻¹
5×10⁻²
10⁻²
5×10⁻³
10⁻³
5×10⁻⁴
10⁻⁴
100
200
300
400
500
600
MINUTES

FIG. 2

DENTAL AND ORAL PREPARATION

FIELD OF THE INVENTION

The invention relates to novel compositions for removing bacteria and other microorganisms from the oral cavity, and especially from the surface of teeth, and removing odor producing materials from oral cavity. The compositions are based on hydrophobic constituents which desorb microorganisms from teeth and other surfaces. The compositions are provided as toothpastes, gels, mouthwash, etc.

BACKGROUND OF THE INVENTION

Tooth decay and periodontal disease are due to bacterial accumulations on the surfaces of teeth in the form of a macroscopic layer generally referred to as dental plaque. Generation of dental caries (cariogenesis) is linked to the presence of certain types of bacteria on the surfaces of teeth. Marginal gingivitis and also certain cases of halitosis have been attributed to the presence and activity of bacteria in the oral cavity.

The most widely practiced treatment of teeth is by brushing with a toothbrush, using certain dentifrices. These generally contain abrasives and detergents. By the mechanical and abrasive action dental plaque is removed from the surfaces of teeth and from crevices. The efficacy of conventional dentrifices is due to the action of the abrasive components and of the detergent ingredients. Such dentifrices may also contain substances such as fluorides and antibacterial agents. The detergents cause foaming, which gives the user a psychological feeling of cleaning action. The use of detergents in the oral cavity also has adverse effects, amongst which there have been reported: gum recession, circulatory impairment, hyperhydration of the exposed tissue, edema, as well as allergic reactions. Detergents are apt to cause stratum corneum swelling, separation between cells as well as increased permeability of human epidermis. It is apparent that the use of less harsh means ought to have an overall beneficial effect.

SUMMARY OF THE INVENTION

There are provided compositions for the removal of microorganisms and especially of bacteria from the oral cavity and from the surface of teeth. The compositions are also effective in the removal of odor-forming constituents and thus for the alleviation of or removal of the effects of halitosis. The compositions contain an active ingredient which removes the microorganisms from surfaces to which they are attached, and which absorb hydrophobic sulfide compounts which cause halitosis. The effective constituent is of a highly hydrophobic nature and a high percentage of adherent oral isolates (about 72 percent) were found to bend avidly to hydrophobic liquids, such as hexadecane, to certain natural oils and the like. As most oral bacteria present in dental plaque and saliva possess pronounced cell surface hydrophobicity, it is possible to effectively remove them by contacting the same with a composition containing such hydrophobic constituent as active ingredient. Amongst active ingredients in the compositions of the invention, there are to be mentioned various types of physiologically acceptable hydrocarbons, vegetable oils and mineral oils. Thus, there are provided according to the invention essentially detergent-free compositions for dental hygiene, such as toothpastes, mouthwashes, and the like, which are based on such oily substances, adapted to effectively desorb, and thus remove, microorganisms from the oral cavity and especially from the surface of teeth.

Experiments have shown that the above defined compounds are also effective for the removal of the unpleasant odor from saliva samples which were allowed to putrify. The odor of halitosis is effectively removed by the compositions of the invention.

The active ingredients of the novel preparations are hydrocarbons and/or fats and/or oils, if desired in combination with aqueous media, or essential oil with antibacterial activity, sweetener, cosmetic additives, preservative, food color and additive such as fluoride or the like. The dentifrice may comprise two phases, an oily one, and an aqueous one, to be mixed just prior to their use.

The high effectivity of hydrocarbons and/or oils of various origin to desorb and bind microorganisms, and especially bacteria of dental plaque, is demonstrated by the following test results.

The compositions of the invention can be effectively used for irrigation of the oral cavity. They can be used for application with irrigation devices such as Water-Pik or the like. The compositions may contain a bacteriostatic or bactericidal agent.

Experiments were carried out in which the ability of the surfaces of teeth to bind hydrophobic bacteria was established. A hydrophobic non-oral bacterium, *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012) and its nonhydrophobic mutant, MR-481 were compared for their adherence to extracted teeth and to human teeth in situ. The hydrophobic RAG-1 cells adhered avidly to the surface of the teeth whereas the MR-481 cells were deficient in their ability to attach to same.

The results presented in Table 1 demonstrate the high effectivity of certain oils in binding bacteria obtained directly from dental surfaces. Amongst oils with a high degree of effectivity there may be mentioned purified olive oil, corn oil and certain hydrocarbons, such as hexadecane and the like.

Experiments were also carried out with test surfaces, such as polystyrene, to which bacteria were bound, and from which these were removed by contacting same with compositions containing as active ingredient olive oil, corn oil or hydrocarbons, such as hexadecane. The high effectivity of removal was established by various techniques, such as staining and by comparing the growth of microorganisms remaining on the test surface after tretment (FIGS. 1 and 2; Tables 1 and 2

TABLE 1

Adherence of dispersed plaque to hydrocarbons[a]		
	% adherence following[b]	
Oil	1st extraction	2nd extraction
hexadecane	23	78
octane	51	87
xylene	73	100
olive oil	48	73

[a]Supragingival dental plaque was collected from a single individual, dispersed by sonic oscillation, washed twice and suspended in saline to yield an initial absorbance of ca. 1.0 at 400 nm, as measured by a Gilford 240 Spectrophotometer. To 1.2 ml of dispersed plaque 0.3 ml of hydrocarbon or olive oil were added and the mixtures mixed uniformly for 120 sec. Following phase separation, absorbance of the lower aqueous phase was measured. The lower aqueous phase was then transferred to a fresh test tube, an additional 0.3 ml of hydrocarbon or olive oil were added, and the phases were again mixed as previously. Following this second extraction, the lower aqueous phase was again removed, and its absorbance recorded.

[b]Results are presented as the percentage decrease in absorbance, compared with that of a sample mixed in the absence of added hydrocarbon or olive oil.

TABLE 2

Desorption of *Acinetobacter calcoaceticus* from polystyrene test tubes by n-alkane

Alkane	Absorbance (arbitrary units)
control (washed in buffer alone)	100
octane	102
decane	103
tetradecane	17
hexadecane	8

TABLE 3

Desorption of *Acinetobacter calcoaceticus* from polystyrene test tubes by edible oils

Oil	Absorbance (arbitrary units)
control (washed in buffer alone)	100
Safflower seed oil	51
Soybean oil	39
Olive oil	33
Coconut oil	15

Legend to Tables 2 and 3: RAG-1 cells were grown overnight to stationary phase in brain heart infusion broth, washed twice and suspended in PUM buffer. One ml of bacterial suspension was added to polystyrene test tubes which were incubated overnight at 37 C. The test tubes were washed twice with PUM to remove nonadherent bacteria. To 1.5 ml PUM buffer in each test tube, 0.3 ml of n-alkane (Table 2) or oil (Table 3) were added and the test tubes vortexed under controlled conditions for 8 min. Following this washing procedure, the mixtures were poured off from the test tubes and the test tubes were washed four times with PUM buffer. Adherent cells were then fixed with methanol and stained with Gentian Violet. Absorbance was measured in a Payton aggregometer fitted with a red filter. Vegetable oils were obtained from Sigma.

Desorption was studied by simple techniques: *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012) cells were grown 18 h to stationary phase in brain heart infusion broth (Difco), conditions which optimize their adherence characteristics. Cells were harvested, washed twice and suspended in PUM buffer (22.2 g $K_2HPO_4.3H_2O$; 7.26 KH_2PO_4; 1.8 g urea, 0.2 g $MgSO_4.7H_2O$ and distilled water to 1000 ml, pH 7.1), to a turbidity of ca. 250 Klett units (measured in a Klett Summerson colorimeter fitted with a green filter). In order to obtain a layer of bound cells, 1.5 ml cell suspension was mixed by vortex for 60 seconds within standard polystyrene cuvettes (Rudolph Brand, Wertheim/Main, FRG). The cell suspension was then poured from the cuvettes. Each cuvette was then washed with 1.5 ml PUM to remove non-bound or poorly attached cells.

To 1.5 ml PUM in each cell-coated cuvette, 0.3 ml olive oil or hexadecane were added, and the mixtures were agitated for 120 seconds by hand. The mixtures were poured off and the process repeated. Following the second mixing, cuvettes were rinsed four times with 1.5 ml PUM to remove as much of the non-aqueous phase as possible from the cuvettes. Control cuvettes were subjected to exactly the same treatment, except that the mixing was performed with 1.5 ml PUM, in the absence of oil.

In order to study desorption of cells qualitatively, cuvettes were fixed with methanol and stained with Gentian Violet. As seen in FIG. **1**, control cuvettes which were washed in the absence of oil maintained a dense layer of attached RAG-1 cells, despite the repeating mixings in the presence of buffer. However, cuvettes washed in the presence of either olive oil or hexadecane appeared completely free of bound cells.

In order to obtain a quantitative estimate of the number of remaining adherent cells, 1.2 ml of minimal medium consisting of PUM supplemented with 0.2% sodium acetate as carbon and energy source, was added to each cuvette. Cuvettes were incubated horizontally at 30° C. Growth was followed turbidimetrically directly in the cuvettes at 400 nm using a Uvikon 710 spectrophotometer (Kontron, Zurich, Switzerland).

Results of a typical experiment are shown in FIG. 2. In the control cuvette, exponential growth was observed following ca. 3 h of incubation. In the cuvette washed with olive oil, growth commenced after 6 h. In both cases doubling time was observed to be the same (50 min). Since an appropriate dilution (1:200) of the RAG-1 cells used in the experiment inoculated into the minimal medium grew following a lag of 100 min (not shown), back extrapolation of the growth curves to 100 minutes provided an estimation of the initial bacterial populations. The extrapolated initial value obtained for the olive-oil washed cuvette (3.5×10^{-4}) was approximately 2.5% of that observed for the control (1.4×10^{-2}). Thus, at least 97% of the bound RAG-1 cells were removed from the cuvette by this procedure, as compared with the control washed in PUM alone. No growth whatsoever was observed in the hexadecane-washed cuvettes during the course of the experiment (9 h), indicating an even higher level of bacterial desorption. Following 20 h incubation, all the cuvettes reached approximately the same turbidity (0.95–1.07), indicating an equivalent growth yield. It should be emphasized that neither hexadecane nor olive oil is toxic for RAG-1 cells; both hexadecane and olive oil can serve as carbon and energy source for growth of RAG-1. The equivalent growth rates measured in the control and olive-oil washed cuvettes provide further evidence that inhibitory effects were not involved in the difference observed, but rather the initial bacterial concentration.

Quantitative comparisons of decorption ability of various n-alkanes and oils were carried out by ascertaining the level of stain remaining on RAG-1 coated test tubes which were washed by aqueous:oil mixtures. Hexadecane (Table 2) and coconut oil (Table 3) were particularly efficient in desorbing bound bacteria.

Qualitative experiments have shown the high effectivity of a mouthwash based on a mixture of 2:1 (v/v) of tap water/olive oil used for swishing the mouth. The mixture was examined and found to contain oil droplets with various forms of oral bacteria adhering to same. The treated surfaces were cleaned in a highly effective manner and only a small percentage of the initial population of microorganisms remained on the surface. Further experiments were carried out by staining dental plaque with erythrosine which specifically stains oral bacteria. Swishing with tap water removed less and less stain. When the swishing was effected with a mixture containing olive oil, a high level of dye was removed together with the plaque, which concentrated at the oil/water interface. As control an aqueous erythrosine solution was mixed with olive oil and no removal of the dye into the oily phase or at the oil/water interface was observed.

The above experiments were corroborated by quantitative clinical trials, performed with four volunteers. In

these experiments, volunteers swished their mouths for seven consecutive thirty-second periods under controlled conditions. The first four swishings were performed with 7.0 ml saline. The two subsequent swishing mixtures consisted either of a 1:1 saline:oil mixture (7.0 ml final volume), or of commercial mouthwash, diluted according to manufacturer's label. The final (seventh) rinse consisted of saline alone. Each volunteer performed a single experiment (seven rinsings) per day.

The amount of biological debris (i.e., bacteria and desquamating epithelial cells) was ascertained quantitatively. Following each swishing, the mixture was expelled from the mouth into centrifuge tubes, and the exact volume recorded. Mixtures were standardized by the following procedure: in experiments comparing oil:saline with saline, the expelled saline rinses were supplemented with 3.5 ml oil, and the oil:water rinses were supplemented with 3.5 ml saline. In this manner, each tube containd ca. 7.0 ml saline+3.5 ml oil. The mixtures were shaken for two minutes. Following this, an equal volume (1 ml) of Gentian violet solution was added to each tube, to an initial concentration corresponding to 1.8–1.9 O.D. at 585 nm, as measured in a Uvikon spectrophotometer. The tubes were again mixed and then centrifuged to clarify the emulsions. Following centrifugation, stain was observed to concentrate both at the oil:aqueous interface, and in the pellet. Microscopic observation revealed that the stained material consisted almost exclusively of oral bacteria and epithelial cells bearing adherent bacteria. Control experiments with different amounts of oral debris demonstrated that a correlation exists between the amount of stain absorbed from the aqueous phase and the amount of oral debris present. The amount of stain absorbed by the oral debris was determined by measuring the concentration of stain remaining in the bulk aqueous phase. This was performed spectrophotometrically by dichromic (585 nm–625 nm) measurement of the bulk aqueous phase. Results obtained spectrophotometrically were corrected for variations in volume of the expelled rinses and expressed as the difference in optical density of the bulk aqueous phase relative to the value obtained for the first rinsing of the same experiment.

Results of the experiments in which four volunteers each performed six experiments (three with oils, three with commercial mouthwashes) is summarized in Table 4. In general, consecutive rinsings with saline (numbers 1–4 in each experiment) removed less and less oral debris, as observed in an increase in optical density of the bulk aqueous phase. However, when the saline was substituted by saline:oil mixtures, removal of higher amounts of oral debris were recorded. Best results were obtained using soya bean oil. The seventh rinse, performed with saline alone, achieved poorer results. The cleansing effect of the oils was not observed in parallel experiments performed with commercial mouthwashes.

These experiments provide direct evidence that rinsing with oil:aqueous solutions in situ has a superior effect in cleansing the oral cavity.

TABLE 4

Relative Concentration of Gentian Violet Remaining in Aqueous Phase following Centrifugation of Expelled Oral Swishes

Volunteer No.	Swish no.	Coconut oil	Olive oil	Soya Bean oil	"Eludril" commerical mouthwash	"Tayadent" commerical mouthwash	"Listermint" commerical mouthwash
1	1	1.0	1.0	1.0	1.0	1.0	1.0
	2	0.98	1.04	1.28	1.28	1.10	1.08
	3	1.07	1.16	1.38	1.33	1.10	1.14
	4	1.11	1.13	1.30	1.37	1.13	1.19
	5	0.74	0.76	0.41	1.38	1.71	1.21
	6	0.75	0.78	0.35	1.42	1.44	1.21
	7	1.06	1.16	1.49	1.27	0.97	1.21
2	1	1.0	1.0	1.0	1.0	1.0	1.0
	2	1.33	1.38	1.29	1.32	1.01	1.11
	3	1.39	1.41	1.40	1.38	1.07	1.08
	4	1.45	1.51	1.63	1.49	1.11	1.15
	5	1.15	1.11	0.79	1.63	1.48	1.10
	6	1.17	0.84	0.79	1.62	1.31	1.12
	7	1.42	1.20	1.36	1.48	1.16	1.03
3	1	1.0	1.0	1.0	1.0	1.0	1.0
	2	1.35	1.11	1.05	1.05	0.99	1.05
	3	1.47	1.07	1.10	1.08	1.02	1.01
	4	1.50	1.38	1.10	1.16	1.08	1.12
	5	1.29	0.98	0.75	1.30	1.54	1.09
	6	1.22	0.98	0.67	1.25	1.39	1.08
	7	1.43	1.30	0.85	1.11	1.11	1.11
4	1	1.0	1.0	1.0	1.0	1.0	1.0
	2	1.17	1.16	1.26	1.15	1.05	1.17
	3	1.22	1.13	1.30	1.25	1.07	1.16
	4	1.22	1.26	1.32	1.29	1.13	1.24
	5	0.96	0.98	0.62	1.46	1.47	1.20
	6	0.92	0.86	0.43	1.45	1.32	1.32
	7	1.22	1.24	1.02	1.22	1.09	1.20

The oily, hydrophobic phase has a two-fold effect: desorption of microorganisms and adsorption of sulfur-containing odorous compounds, such as mercaptans which form by the action of bacteria on salivary proteins.

The novel composition can be applied by squirting into the mouth, swishing around for a while and ejection; they may be applied by means of a toothbrush, which enhances the effect by adding the mechanical effect of plaque removal.

Further experiments demonstrated the ability of hydrocarbns and different oils to remove odor producing materials from the oral cavity. Halitosis in vivo is caused mainly by stasis of saliva in different microniches in the oral cavity especially during the night,

resulting in production of odorous sulphides e.g. methyl mercaptan, dimethyl sulphide, ethyl mercaptan.

In a typical experiment saliva was collected from a volunteer and equally divided in two test tubes. One ml of soybean oil was added to one of the test tubes and both were equally mixed for 60 seconds. Following phase separation the lower aqueous phase from the experiment test tube was transferred to a fresh test tube. The experimental fresh test tube and the control were incubated in 37° C. for 24 hours allowing the saliva to putrify.

Putrified saliva samples were then diluted 1:10 in P.B.S. (phosphate Buffered Salin) and examined organoleptically by five independent volunteers. All the examiners claimed of definite difference between the experimental sample and the control. The saliva sample that was mixed with oil prior to incubation exhibited slight or no unpleasant odor while the control sample had a strong foul odor. In an additional experiment, collected saliva was first allowed to putrify under the same conditions. One half of the saliva volume was mixed for 60 seconds in the presence of soybean oil and the other half served as control. Following phase separation the lower aqueous phase was transferred to a new test tube. Similar volumes of the experimental and the control samples were diluted 1:10 with P.B.S. and again examined organoleptically by five independent volunteers.

Slight or no odor was reported when smelling the experimental sample while strong foul odor was smelled form the control sample.

The following examples are intended to illustrate the invention, and these are to be construed in a non-limitative manner.

In the following examples percentages are by weight. The compositions were prepared by conventional techniques which do not require any detailed description.

Example 1 - High viscosity mouthwash	
Olive oil	52%
Coconut oil	9.9%
Corn oil	35%
BHTA (anti-oxidant)	0.1%
Orange oil	3%
Example 2 - Vitamin containing toothpaste	
White mineral oil	36.0%
Olive oil	33.0%
Coconut oil	12.0%
Talc	18.0%
Orange Oil	0.9%
Vitamin A	.01%
Vitamin D_3	.01%
Vitamin E	.1%
Example 3 - Low viscosity mouthwash	
Decane	28.9%
Dodecane	38.0%
Orange terpenes	5.0%
Low MW silicon oil	27.0%
BHTA	0.1%
Orange oil	1.0%
Example 4 - Toothpaste	
Talc	10%
Kaolin	13%
Beeswax	8%
Carnauba wax	3%
White mineral oil	43%
Olive Oil	19%
Anise oil	2%
Cocoa butter	1.8%
Pigment red 57	0.2%
Example 5 - Abrasive-free Lotion for Toothbrushing	
Ethyl cellulose	9.5%
Mineral oil	40.0%
Olive oil	40.0%
Orange terpenes	5.0%
BHTA	0.1%
Beta carotene	0.2%
Saccharin	0.2%

Example 6 - Two color biphasic combination toothpaste (1:1 ratio) containing fluoride

Oil phase:		Water phase:	
white mineral oil	20%	alginate	10.0%
Olive oil	20%	Saccharin	0.2%
Beeswax	35%	Water	88.8%
Talc	10%	Sodium mono-	0.5%
Corn oil	13%	fluorophosphate	
Lemon oil	1.8%	erythrosine	0.5%
Beta carotene	0.2%		

EXAMPLE 7

A two-phase mouthwash was prepared comprising an aqueous 0.85% solution of sodium chloride in water and half the volume of oily phase: soya bean oil.

The composition was provided in a double-compartment double-squirt bottle and allowed the squirting of a small quantity (a few milliliters) of the mixture of the two ingredients into the oral cavity.

Results indicate that the composition is effective in removing dental plaque, a large percentage of adhering microorganisms and that it also reduced to a large extent halitosis.

LEGENDS TO THE FIGURES

FIG. 1: Cuvettes were washed and rinsed, as described in the test. The remaining adherent cells were visualized by fixation in methanol and staining with Gentian Violet. Left-control cuvette, washed with PUM alone; Center-cuvette washed with olive oil; right-cuvette washed with n-hexadecane.

FIG. 2: RAG-1 cells bound to polystyrene were desorbed and rinsed as described in the text. To each cuvette, 1.2 ml acetate medium (PUM supplemented with 0.2% acetate) was added. Outgrowth of cells was measured turbidimetrically. Results are back extrapolated back to the end of the lag period (100 min). (0), washed with olive oil:PUM mixture; (0), washed with PUM alone.

We claim:

1. A mouthwash for dental and oral hygiene, adapted to desorb microorganisms and absorb odorous mercapto compounds causing halitosis, which mouthwash comprises a system of two physically separate liquid phases, provided in a double compartment doublesquirt bottle adapted to be emulsified by swishing in the mouth, one of said phases being a solution of sodium chloride in water as an aqueous phase, and the other an oily phase which is a vegetable oil, a mineral oil or a hydrocarbon, said aqueous phase and said oily phase being in the ratio between 2:1 and 1:1 by volume.

2. A mouthwash according to claim 1 containing a bacteriostatic or bactericidal agent.

3. A mouthwash according to claim 1 containing also an essential oil.

4. A mouthwash according to claim 1, wherein the oily phase is a member selected from the group consisting of olive oil, corn oil, soybean oil, safflower oil, coconut oil and paraffin oil.

5. The method of removing microorganisms, odorous substances from the oral cavity which consists of irrigating the oral cavity with the mouthwash composition in accordance with claim 1 squirted as a mixture comprising an aqueous phase and an oily phase, in the ratio between 2:1 and 1:1 by volume.

* * * * *

File: 1987-us-patent-4687746.pdf

United States Patent [19]

Rosenberg et al.

[11] **Patent Number: 4,687,746**

[45] **Date of Patent: Aug. 18, 1987**

[54] **MICROORGANISM CULTURE-TRANSFER DEVICE**

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[73] Assignee: **Ramot University Authority for Applied Research and Industrial Development Ltd.**, Tel Aviv, Israel

[21] Appl. No.: **690,999**

[22] Filed: **Jan. 14, 1985**

[30] **Foreign Application Priority Data**

Jan. 20, 1984 [IL] Israel 70737

[51] **Int. Cl.**[4] **C12M 1/26**

[52] **U.S. Cl.** **435/292**; 128/759; 422/100

[58] **Field of Search** 435/292, 294, 295; 81/177.1, 177.3; 128/757, 756, 759, 304; 73/864.72; 422/68, 100

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Primary Examiner—Alan Cohan

Attorney, Agent, or Firm—Benjamin J. Barish

[57] **ABSTRACT**

Microorganism culture transfer devices are described of the type comprising a manually-grippable handle having a culture contacting tip for contacting a culture and streaking same a plurality of times over a culture plate in order to dilute the culture for purposes of isolating individual colonies. The handle is of a configuration in cross-section defining at least three, preferably four or more, discrete rotational positions when held and rotated by the user's fingers about the longitudinal axis of the handle; and the culture-contacting tip is of a configuration such as to provide a different and distinct surface for contact with the culture in each of the discrete rotational positions of the handle.

9 Claims, 12 Drawing Figures

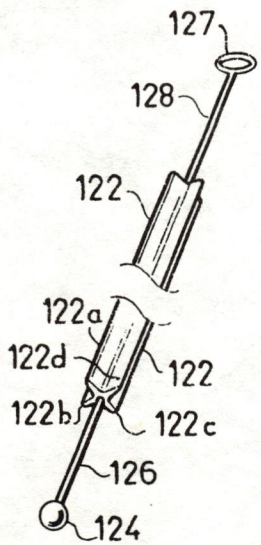


FIG 1

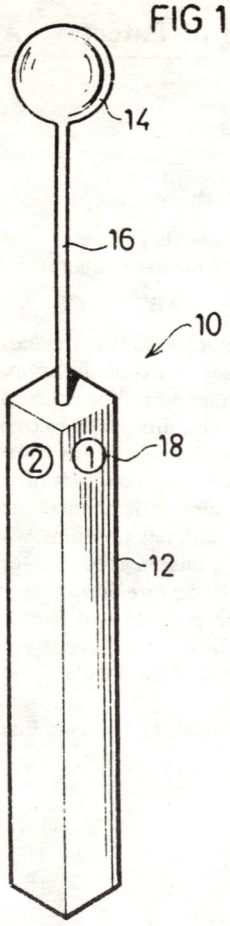
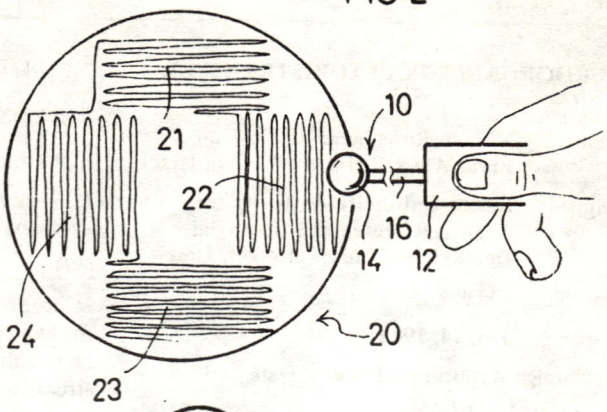


FIG 2



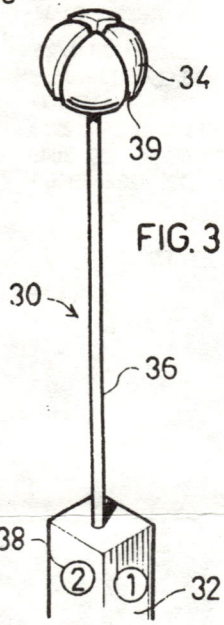


FIG. 3

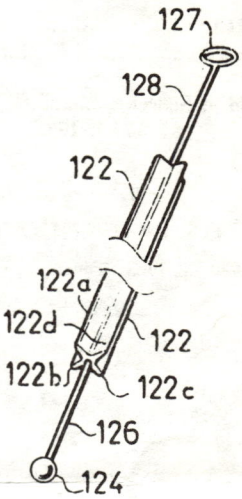


FIG.12

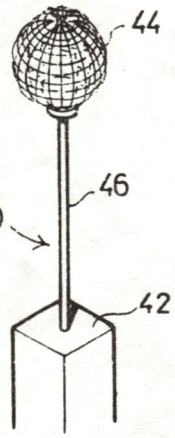


FIG 4

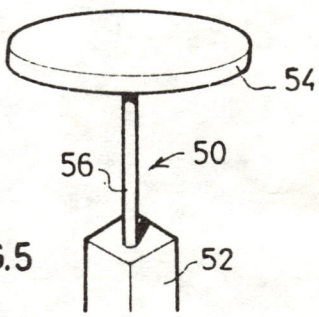


FIG.5

FIG. 6

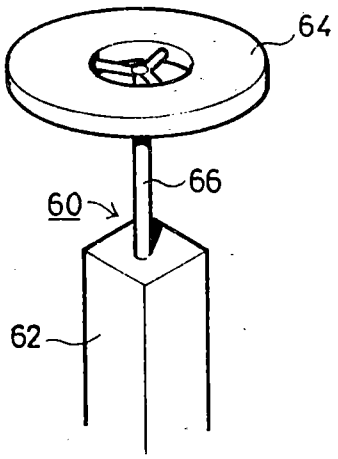


FIG. 8

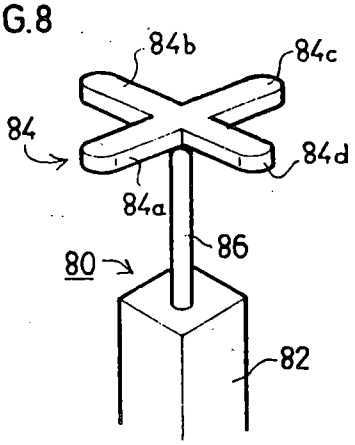
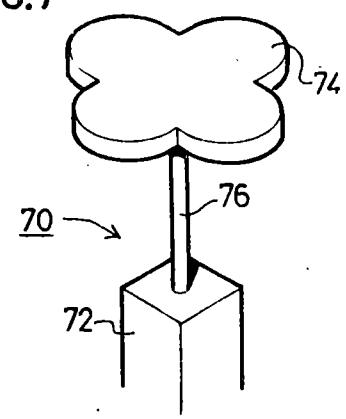


FIG. 7



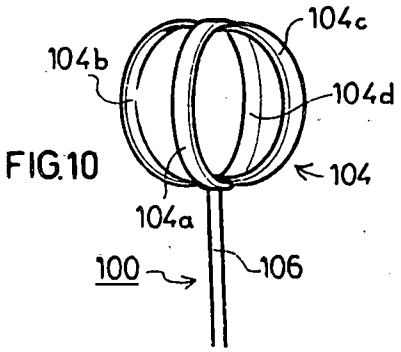


FIG. 10

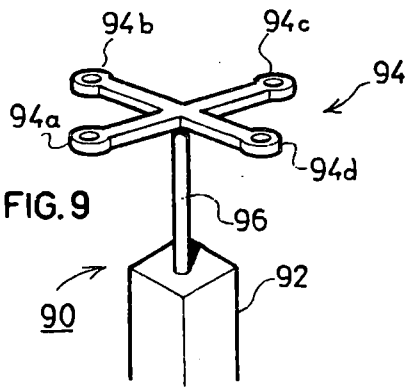


FIG. 9

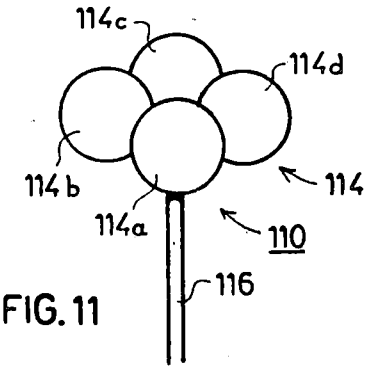


FIG. 11

MICROORGANISM CULTURE-TRANSFER DEVICE

BACKGROUND OF THE INVENTION

This invention pertains to the general field of microbiology, and more specifically to a microorganism culture-transfer device for transferring microorganisms to and from solid media for the purpose of obtaining individual colonies.

Most microbiological laboratories must maintain pure cultures of microorganisms for reference and research. In order to maintain strains of individual microorganisms, the cultures are usually streaked onto plates containing solidified medium. The inoculum of the culture is generally from a single colony on a previously inoculated plate, and the aim of the streaking procedure is to obtain individual colonies of the strain on the fresh plate. Individual colonies of identical form signify to the worker that a single cell type has been transferred; moreover a single colony serves as the inoculum for further strain transfer and experimentation. The transfer technique is generally performed at present with a loop at the end of a long handle. The traditional loop is formed from metal (e.g. platinum or nickel-chromium) which can be quickly heat sterilized (e.g. by flame) and quickly cooled by touching the sterile surface of the fresh medium. Recently, sterile, plastic single-use, disposable loops have been introduced.

In the classic technique, known as streaking, the sterile loop is first brought into contact with the colony whose cells one wishes to transfer; it is then brought into contact with the plate containing sterile solid medium to which one wishes to transfer the cells, and is moved back and forth over the surface of a portion (e.g. one quarter) of the fresh plate. In the initial step, millions of cells may be transferred to the new plate, and must be further diluted by many orders of magnitude so that individual colonies, rather than confluent growth, will be obtained. Since the loop surface is also now contaminated with many thousands of cells, it must be either resterilized (e.g., by flame sterilization in the case of wire loops), or substituted by a new sterile loop (e.g. in the case of single-use, disposable plastic loops). The sterile loop is then brought into contact with the surface of the fresh plate which has been previously streaked, e.g. by several movements across the zone of the first streaking, and then another portion of the plate (e.g. a quarter of the plate adjacent to the first streaking) is streaked. This results in additional dilution of the cells. The loop is then either resterilized or replaced, is brought into contact with the second streaking area, and then a third zone of the plate is streaked. This process must be performed at least three or four times in order to be certain of obtaining individual colonies, rather than confluent growth, following incubation of the fresh plate.

BRIEF SUMMARY OF THE INVENTION

An object of the present invention is to provide a microorganism culture-transfer device having advantages over the known devices in the above respects.

According to a broad aspect of the present invention, there is provided a microorganism culture-transfer device comprising a manually-grippable handle having a culture contacting tip for contacting a culture and streaking same a plurality of times over a culture plate in order to dilute the culture for purposes of isolating individual colonies, characterized in that the handle is of a configuration non-circular in cross-section having a plurality of at least three longitudinally-extending linear surfaces projecting from the outer face of the handle defining at least three discrete rotational positions when held between two of the user's fingers and rotated about the longitudinal axis of the handle, by moving one of the fingers relative to the other, the culture-contacting tip being of a configuration such as to provide a different and distinct surface for contact with the culture medium in each of said discrete rotational positions of the handle.

The novel device constructed in accordance with the foregoing features provides a number of important advantages over the known devices briefly described above. Thus, when the known wire loop is used, it is necessary to heat and cool the wire loop between each streaking operation, there being typically four streaking operations to obtain the required colony dilution. When the disposable loop is used, a new loop must be brought to the culture medium for each streaking operation, and the previous loop discarded. Both cases involve not only significant time and effort, but also some chance of contaminating either the culture plate or the loop with foreign microorganisms, because of the many manipulations required and the duration that the culture plate is required to be open. Such time, effort, and chances of contamination are all considerably reduced with the present invention since the same device is used for all the streaking operations and is merely rotated a partial revolution (e.g. one-fourth revolution for performing four streaking operations) between each streaking operation.

Another important advantage, particularly when the disposable device is used, is that all the streaking operations require only one device rather than a number (e.g. four) required when using the known disposable loops.

The novel device may also be employed for inoculation from liquid medium to solid medium, from various solid surfaces, or for inoculation of mixed cultures, in a manner similar to that employed by presently existing devices.

The culture-contacting tip of the novel device may take a number of configurations, several of which are described below for the purposes of example. Thus, described below are tips having the configurations of a sphere, a disc, an annular ring, a clover leaf, and a plurality of radiating ribs, intersecting loops, or spheres.

The handle may be of polygonal cross-section (e.g. square), formed with a plurality of planar sides, the junctures between adjoining sides of the handle constituting the above-mentioned longitudinally-extending linear surfaces projecting from the outer face of the handle, such that each side defines one of the plurality of discrete rotational positions of the handle; alternatively, the handle may be formed with longitudinally-extending ribs constituting the above-mentioned longitudinally-extending linear surfaces projecting from the outer face of the handle and defining the discrete rotational positions. Markings may be provided on the handle to indicate a particular sequence of use, e.g. raised dots, or numbers, or different coloured markings for each side of the handle.

The device of the present invention may be constructed either as a reusable unit, wherein the culture-contacting tip may of metal, ceramic, wood, autoclavable plastic or the like; or it could be constructed as a

one-time, disposable unit, wherein the device may be of plastic, wood or the like.

Further features and advantages of the device will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. **1** is a three-dimensional view illustrating one form of microorganism culture-transfer device constructed in accordance with the present invention;

FIG. **2** illustrates how the device of FIG. **1** is used for transferring a culture by a plurality of streaking operations in order to isolate a single colony;

FIGS. **3–11** are fragmentary views illustrating other configurations of culture-transfer devices in accordance with the present invention; and

FIG. **12** illustrates a still further device in accordance with the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

FIG. **1** illustrates a culture-transferring device **10** including a handle **12** at one end, and a culture-contacting tip **14** at the opposite end joined to the handle by a stem **16**. In the embodiment of the invention illustrated in FIG. **1**, handle **12** is of square cross-section, and the culture-contacting tip **14** is of spherical configuration, having a diameter, e.g., of 0.2–1.0 cm. When device **10** is constructed for reuse, it may be constructed of autoclavable material, such as polypropylene, polycarbonate, wood, ceramic, metal or the like, or a combination thereof. When device **10** is constructed for one-time use, it may be of plastic (e.g. polystyrene), wood or the like, presterilized by irradiation.

FIG. **2** illustrates the manner of using the device **10** in a plural-streaking operation in order to dilute a culture sufficiently for isolating individual colonies. The culture medium is in a Petri dish **20**. In the dilution procedure, device **10** is used first to streak region **21** of the culture medium, and then regions **22**, **23**, **24**, in that sequence, so that the culture in the final region **24** will be diluted several orders of magnitude with respect to the culture in the first region **21**.

When device **10** is used in performing this sequence of streaking operations, its handle **12** is grasped by the user's fingers and is rotated above the longitudinal axis of the device one-fourth revolution between each streaking operation in order to provide a different surface for contact with the culture during each such streaking operation.

Thus, to produce the streaking operation in the first region **21**, the user holds device **10** in one rotational position, brings the undersurface of tip **14** into contact with the colony of cells to be transferred, brings the same undersurface of tip **14** into contact with the culture medium **20** and performs the streaking operation in the first region **21**. Then the user, while still holding the device proximally to the culture medium, rotates it one-fourth revolution about its longitudinal axis to present a different and distinct surface of tip **14** for contact with the culture medium **20**, and performs the second streaking operation in region **22** of the culture medium. The device **10** is then rotated by the user another one-fourth revolution to present a third fresh surface of tip **14** for contact with the culture medium, and performs the third streaking operation in region **23**; and then rotates the device another one-fourth revolution to present a fourth fresh surface of tip **14** for contact with the culture to perform the fourth streaking operation in region **24** of the culture medium.

The square cross-section of handle **12** greatly facilitates the rotation of handle **12**, and thereby of tip **14**, one-fourth revolution between each streaking operation, this rotation being effected by gripping the handle between the user's thumb and index finger and then shifting the thumb laterally with respect to the index finger. Preferably, the four faces of handle **12** are sequentially numbered "1–4", as shown by indicia **18** in FIG. **1**, or provided with other indications to inform the user of the proper sequential rotation of the device.

FIGS. **3–11** illustrate further configurations of the culture contacting tip which may be used in substantially the same manner as described above with respect to the device of FIG. **1**. In all these variations, the handle is illustrated as of square-cross-section, since such devices are typically used for performing four streaking operations. It will be appreciated, however, that the cross-section of the handle could include a different number of sides (e.g., three or five) if the device is to be used in a procedure involving three or five streaking operations, respectively.

Thus, the device illustrated in FIG. **3**, and therein generally designated **30**, includes a handle **32**, a spherical culture-contacting tip **34**, a stem **36**, and indicia **38**, all as described above with respect to device **10** illustrated i FIG. **1**. The only difference in the device illustrated in FIG. **3** is that the spherical tip **34** is formed with two circular recesses **39** over its outer face dividing its outer face into four discrete surface sections for contact with the culture medium, each section being separated by a portion of he circular recess **39**. Device **30** of FIG. **3** may be constructed for reuse, in which case the circuit tip **34** would be of metal for flame-sterilization or of wood for autoclaving; or it could be constructed for one-time use, in which case tip **34** would be of plastic, wood, ceramic or the like, sterile or sterilizable, e.g. by irradiation.

FIG. **4** illustrates a construction particularly useful when the device is for reuse. Thus, the device **40** also includes a handle **42**, a spherical tip **44**, and a stem **46**, except in this case the spherical tip **44** is made of a mesh of metal wire to facilitate sterilization by flame before the device is to be used for performing the first streaking operation; its sterilization between streaking operations in the same isolation procedure is not required as when using the conventional metal loop.

FIG. **5** illustrates another device **50**, also including a handle **52**, a culture-contacting tip **54**, and a stem **56**, except in this case the culture-contacting tip **54** is in the shape of a circular disc fixed to the handle **52** perpendicularly to the longitudinal axis of the handle. That is, the planar axis through disc **54** is perpendicular to the longitudinal axis of handle **52**. Device **50** illustrated in FIG. **5** is used in the same manner as described with respect to FIG. **2** since, in practice, only the annular equatorial portion of the spherical tip **14** in FIG. **1** is actually used, this portion being equivalent to the outer edge of disc **54** in FIG. **5**.

FIG. **6** illustrates a still further variation wherein the culture-contacting tip, therein designated **54**, is in the shape of an annular ring, equivalent to the outer portion of disc **54** in FIG. **5**. As in FIG. **5**, the plane of ring **64** in FIG. **6** i also fixed, via stem **66**, to the handle **62** so

as to extend perpendicularly to the longitudinal axis of the handle.

FIG. 7 illustrates a still further variation **70** wherein the culture-contacting tip **74** is in the configuration of a clover leaf having four sections, one for each of the four sides of the handle **72**. The clover leaf tip in FIG. **7** is also secured, via stem **76**, to extend perpendicularly to the longitudinal axis of handle **72** so as to provide a separate fresh surface for contact with the culture medium during each rotational position of the handle.

FIG. **8** illustrates a still further variation **80** wherein the culture-contacting tip **84** is constituted of a plurality of ribs **84***a*–**84***d* joined together at their inner ends to handle **82**, via stem **86**, and radiating outwardly of the handle in a direction perpendicular to the longitudinal axis of the handle. Thus, the outer tip of each of the radiating ribs **84***a*–**84***d* is positioned to contact the culture medium with each one-fourth rotation of the handle during the procedure described above with respect to FIG. **2**.

FIG. **9** illustrates a variation in the rib construction of FIG. **8**, wherein the culture-contacting tip **94** secured to handle **92** of the device **90** by stem **96** is also formed with radiating ribs **94***a*–**94***d*, but the outer tip of each rib is formed with a loop for contact with the culture medium.

FIG. **10** illustrates a still further variation wherein the culture-contacting tip **104** at the end of stem **106** of the device **100** is in the configuration of two intersecting loops, thereby providing four semi-circular surface **104***a*–**104***d*, each separate and distinct from the others, for contact with the culture medium during the four streaking operations.

FIG. **11** illustrates a still further variation wherein the culture-contacting tip **114** carried at the end of stem **116** of the device **100** is in the configuration of four contiguous spheres **114***a*–**114***d*, preferably joined together along the longitudinal axis of the device.

FIG. **12** illustrates a culture-transfer device comprising a manually-grippable handle having a spherical-type culture-contacting tip **124** at one end, and a conventional loop **127** at the opposite end. Thus, the spherical tip **124** may be of the same construction as tip **14** in FIG. **1** and is joined to the respective end of handle **122** by a stem **126**. The conventional loop **127** is joined to the opposite end of handle by another stem **128**. In this case, handle **122** is provided with a plurality of radiating ribs **122***a*–**122***d* to define the plurality of discrete rotational positions of the handle when held by the user's fingers and rotated about the handle longitudinal axis. In the FIG. **12** arrangement, handle **122** is of X-cross-section, defining four radiating ribs **122***a*–**122***d*, but it will be appreciated that the handle could include a different number of such ribs to define a different number of discrete positions of the culture-contacting tip **124**.

Preferably, the device illustrated in FIG. **12** is made as a unitary device of plastic material, the X-section of the handle providing the additional advantage of permitting faster cooling after molding because of its increased outer surface area as compared to that of the polygonal configuration of the other embodiments.

The spherical tip **124**, as well as the culture-contacting tips in the other described arrangements, may be provided with a roughened surface.

Many other variations, modifications and applications of the invention will be apparent.

What is claimed is:

1. A microorganism culture transfer device comprising a manually-grippable handle having a culture contacting tip at its two opposite ends for contacting a culture and streaking same a plurality of times over a culture plate in order to dilute the culture for purposes of isolating individual colonies, characterized in that said handle is of a configuration non-circular in cross-section having a plurality of at least three longitudinally-extending ribs constituting linear surfaces projecting from the outer face of the handle defining at least three discrete rotational positions when held between two of the use's fingers and rotated about the lingitudinal axis of the handle by moving one of said fingers relative to the other; and in that at least one of said culture-contacting tips is of a configuration such as to present a surface for contact with the culture when the handle is initially grasped by the user in any rotational position of the handle, and to provide a different and distinct but like surface around its circumference for contact with the culture in each of said discrete rotational positions of the handle.

2. The device according to claim **1**, wherein said one culture-contacting tip is of spherical configuration.

3. The device according to claim **2**, wherein the outer surface of said spherical culture-contacting tip is formed with a plurality of circular recesses over its outer face dividing it into discrete surface sections for contact with the culture.

4. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of a circular disc fixed to the handle perpendicularly to the longitudinal axis of the handle.

5. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of an annular ring fixed to the handle perpendicularly to the longitudinal axis of the handle.

6. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of a clover leaf fixed to the handle perpendicularly to the longitudinal axis of the handle, which clover leaf has a separate section for each of said rotational positions of the handle.

7. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of a plurality of ribs, one for each of said rotational positions of the handle, which ribs are joined together and to said handle at their inner ends and radiate outwardly of said handle in a direction perpendicularly to the longitudinal axis of the handle.

8. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of a plurality of intersecting loops to provide a plurality of different and distinct surfaces for contact with the culture.

9. The device according to claim **1**, wherein said one culture-contacting tip is in the configuration of a plurality of contiguous spheres joined together along the longitudinal axis of the device.

* * * * *

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CERTIFICATE OF GRANT OF PATENT

In accordance with Section 24(2) of the Patents Act, 1977, it is hereby certified that a patent having the specification No 2219937 has been granted to Ramot University Authority for Applied Research and Industrial Development Ltd, in respect of an invention disclosed in an application for that patent having a date of filing of 22 March 1989 being an invention for "Composition for desorbing bacteria"

Dated this Sixth day of November 1991

P.R.S. HARTNACK

Comptroller-General of Patents, Designs and Trade Marks.

UNITED KINGDOM PATENT OFFICE

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THE ATTENTION OF THE PROPRIETOR(S) IS DRAWN TO THE IMPORTANT NOTES OVERLEAF

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IMPORTANT NOTES FOR PROPRIETORS OF UNITED KINGDOM PATENTS

1. DURATION OF PATENT & PAYMENT OF RENEWAL FEES

(i) A patent takes effect on the date shown at the foot of the certificate overleaf. Subject to the payment of renewal fees, it can be kept in force until the end of a period of 20 years from the date of filing the patent application.

(ii) To maintain the patent in force, it is necessary for the proprietor or someone on his behalf to pay a prescribed annual renewal fee. Payment may be made on, or during the three month period before, the fourth or subsequent anniversary of the date of filing the application and should be accompanied by Patents Form 12/77.

(iii) **The proprietor is responsible for ensuring that effective renewal arrangements are set up and maintained and that fees are paid on time.** He should not await any communication from Patent Office before paying the fee; an official reminder sent to the last recorded address for service within six weeks **after** the anniversary is intended to alert the proprietor to possible failure of his renewal arrangements.

(iv) If the form with the fee is not lodged in the Patent Office on or before the anniversary of the filing date of the patent, the fee cannot be accepted unless application for an extension of time to a maximum of 6 months is made and paid for on Patents Form 12/77. If no renewal fee is received and no extension of time is requested, the patent will cease. No reduction of extension fees is made in the case of a patent endorsed "Licences of Right". When paying a renewal or extension fee it is advisable first to check the current scale of charges as these may change from time to time.

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Patents Forms, together with the fees and fee sheet (FS. 1) should be delivered to the Patent Office in Cardiff Road, Newport either by hand or post; those sent by post should be addressed to "The Cashier, The Patent Office, Cardiff Road, Newport, Gwent, NP9 1RH". Alternatively, they may be delivered by hand to "The Patent Office, 25 Southampton Buildings, London, WC2A 1AY".

Blank Patents Forms and fee sheets (FS. 1) can be obtained by post from the Clerk of Stationery, The Patent Office, Unit 6, Nine Mile Point, Cwmfelinfach, Newport, Gwent, NP1 7HZ or may be collected from the above Newport or London address.

3. REGISTRATION OF OWNERSHIP AS EVIDENCE OF ENTITLEMENT

Any person who claims to have acquired the property in a patent by virtue of any transaction, instrument or event shall be entitled as against any other person who claims to have acquired that property by virtue of an earlier transaction, if application is made to the comptroller for registration in the register of patents (see Sections 32 and 33 of the Patents Act 1977). Details of how to make such application may be obtained from the Patent Office.

COMPOSITION FOR DESORBING BACTERIA

The present invention relates to novel compositions which desorb bacteria and other microorganisms from solid surfaces and from living tissue.

The main use contemplated for the compositions of the present invention is in oral hygiene. Compositions of the invention can also be used in entirely different applications, where microorganisms are to be removed from surfaces to which they are attached, or where enhanced microbial adhesion to oil droplets is desired.

According to the present invention there is now provided a composition for desorbing bacteria from solid surfaces and from living tissues, which is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime said composition comprising:

a) from 50 to 97% w/w of an aqueous phase;

b) from 3 to 50% w/w of a water immiscible oily phase, comprising a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydrocarbon or a mixture thereof; and

c) from 0.003 to 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

Amongst edible oils, which can be used to form said water immiscible oily phase there may be mentioned oils such as olive oil, corn oil, coconut oil, soybean oil, safflower oil. There can also be used a wide variety of pharmacologically acceptable hydrocabons such as octane, decane, tetradecane, hexadecane, xylene, white mineral oil, and mixtures thereof etc.

Amongst suitable colors, there may be mentioned colors of triphenylmethane, naphthol, xanthene, monoazo, pyrazol, anthraquinone and cationic colors; examples include Food Blue 2 and its ammonium salt; D&C Yellow No. 7, D&C Yellow No. 10, D&C Yellow Nos. 4, 6, 22, 28, 33 or 40; D&C Green No. 5, D&C Orange No. 11, D&C Red Nos. 19 and 37, Basic Blue Nos. 6, 9, 41, 99, etc.

Preferably said amphipathic cationic moiety is selected from surface-active cationic molecules containing a pyridinium moiety such as cetylpyridinium chloride, laurylpyridinium chloride, etc.; from chlorhexidines such as chlorhexidine, its diacetate, chlorhexidine digluconate, chlorhexidine dihydrochloride; from monalkyl quaternary ammonium compounds (quats) such as benzalkonium chloride, cetalkonium chloride and bromide, lauralkonium chloride and bromide, soytrimonium chloride, PEG-5 stearyl ammonium lactate; from dialkyl quaternary ammonium compounds (diquats) such as dilauryl dimonium chloride, dicetyl dimonium chloride and bromide, dequalinium chloride, soyamido propyl benzyldimonium chloride, quaterniums such as quaternium 15 and

polyquaterniums, etc; amine fluorides, from cationic polysaccharides, such as chitosan and its derivatives; from cationic polypeptides, such as poly L-lysine, poly D-lysine, lysozyme.

In especially preferred embodiments of the present invention said amphipathic cationic moiety is selected from cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme, particularly from 0.005 to 0.5% cetylpyridinium chloride optionally with 0.005 to 0.5% of a chlorhexidene compound.

The invention also provides a method for enhancing desorption of microorganisms for host industrial and environmental media onto oil droplets, the method comprising admixing an aqueous microbial suspension and oil in the presence of an amphipathic cationic moiety.

In U.S. Patent No. 4,525,342, Weiss et al. have described an essentially detergent free mouthwash based on a combination of an oily phase and an aqueous one. This composition must be marketed in a two-compartment squirt bottle, and the user swishes the two phases in his mouth in order to attain the desired effect. The composition is based on the attachment of amphipathic substances to the oil droplets during the swishing action, thus coating the oil droplets. The presence of detergents is reported to be deleterious in said patent. Various deleterious physiological effects have been reported regarding the use of detergents in te oral cavity. It has been reported that the presence of detergents prevents, or at least decreases adhesion of

microorganisms to oil droplets as is taught, e.g. by the statement: "The aqueous bacterial suspension must be free of surfactants, which inhibit the assay" (M. Rosenberg, 1984. Bacterial Adherence to Hydrocarbons: A Useful Technique for Studying Cell Surface Hydrophobicity. FEMS Microbiology Letters 22, 289-295). In a recent review on hydrophobic interactions and adhesion, the authors write: "Our appreciation of the extent to which hydrophobic interactions mediate various bacterial adhesion phenomena could be greatly augmented by including agents that interfere with hydrophobic interactions (e.g., surfactants, chaotrophic agents) in adhesion assays (M. Rosenberg and S. Kjellerberg, 1986, Hydrophobic Interactions: Role in Bacterial Adhesion. Advances in Microbial Ecology 9, 353-393). Moreover, in a previous study which did examine the effect of some cationic amphipathic antibacterial agents on adhesion of relatively non-hydrophobic Escherichia coli strains to xylene, the cells were exposed to the cationic agents, but the cationic agents were subsequently removed by washing the cell pellet prior to addition of the xylene, presumably to avoid inhibition of the adhesion in the presence of the cationic agents (B.M.A. El-Falana, D.T. Rogers, A.D. Russell and J.R. Furr. 1985 Effect of Some Antibacterial Agents on the Hydrophobicity of Wild-Type and Envelope Mutant of Escherichia coli. Current Microbiology 12, 187-190). Similarly, chlorhexidine, an amphipathic, surface-active cationic agent commonly incorporated into mouthwashes has been shown in many studies to inhibit microbial adhesion to surfaces. For example, J.

McCourtie, T.W. MacFarlane and L.P. Samaranayake (Effect of saliva and serum on the adherence of Candida species to chlorhexidine-treated denture acrylic, J. Med. Microbiology, 21:209-213, 1986) showed that treatment of saliva- or serum-coated acrylic with chlorhexidine gluconate reduced adherence by between 19 and 86%.

Moreover, whereas salts such as sodium chloride have been generally considered to enhance microbial adhesion to surfaces, it has now been found that the presence of inorganic cations, such as sodium and magnesium cations, inhibit the stimulation of adhesion brought about by cationic moieties such as cetylpyridinium, chlorhexidine, chitosan, poly L-lysine, poly D-lysine and lysozyme.

The main use of the compositions is in the form of mouthwashes, which effectively remove a large percentage of microorganisms, debris and other odor-causing materials from the surface of teeth and from the oral cavity. Thus especially preferred embodiments of the present invention relate to a mouthwash as defined above comprising about 0.003 to about 0.5 w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

The novel compositions comprise, in combination, an organic water-immiscible phase and an aqueous phase, and a small quantity of an amphipatic cationic moiety, which is adequate to form upon vigorous mixing an emulsion of brief life-time. In addition, the composition may comprise additional cationic agent or agents which enhance adhesion to oil droplets, as shall be illustrated later. Optimally, the composition should be relatively free from interfering cations, such as sodium, potassium and magnesium and therefore preferably contain only up to 0.3% of inorganic salts.

In use, the two phases are mixed by shaking, and an emulsion is formed which is used for swishing in the mouth, and which has a limited life time, of the duration of about 10 seconds to about 30 minutes. A small amount of amine fluoride which tends to stabilize the emulsion for a brief period of time and has other beneficial (e.g. anticaries, antibacterial) effects may also be added.

There may also be incorporated certain additives such as fragrances, colors and the like.

While the invention will now be described in connection with certain preferred embodiments in the following examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives,

modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

The following experimental results illustrate the invention, both in regard to the specific formulations and also as regards the mechanism of action of the emulsions of the present invention as regards removal of dental plaque and adhering bacterial and other microorganisms .

The mouthwash compositions of the invention preferably comprise a small quantity of an amphipatic cationic moiety which is preferrably a surface-active agent which is able to form an emulsion of limited life time. The emulsion ought to be formed upon shaking of the container which contains the two separate phases, and it should preferably remain in emulsion form for at least about 20 seconds. Experiments have shown that compositions of the invention form temporary emulsions which separate after a period of about 10 seconds to 10 minutes.

We have found the unexpected result that small amounts of cationic surface-active agents promote, rather than inhibit, adhesion of bacteria and oral debris, to oil droplets. We have also found the surprising and unexpected results that sodium and magnesium chloride inhibit, rather than enhance, microbial adhesion in the presence of such cationic agents as cetylpyridinium chloride, chlorhexidine digluconate and chitosan.

The following experiments demonstrate the ability of the cationic surfactant, cetylpyridinium chloride, to enhance microbial adhesion to oil droplets in various experiments. In one experiment Acinetobacter calcoaceticus RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30°C in brain heart infusion broth. The cells were harvested by centrifugation and washed twice in 0.2% saline. The cells were then suspended in 0.2% saline to a corrected optical density of about 15 at 400 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% detergent in water (or water for control) and 0.24 ml water. Following brief mixing, 0.2 ml of oil was added (vs. controls with no oil added) and the mixtures vortexed vigorously for 2 min. Following about thirty minutes, the corrected turbidity of the lower aqueous phase was measured at 400 nm. Adhesion is calculated as the percent drop in turbidity following the mixing procedure. The results are summarized in the following Table.

Table 1. Adhesion RAG-1 to various oils in the presence of cetylpyridinium chloride

Additions	Percent adhesion to:		
	n-nexadecane	mineral oil 50	soybean oil
Cetylpyridinium chloride	83	80	47
None	27	5	32
Sodium dodecyl sulphate	0	0	0
Tween 20	0	0	0

As can be seen from the above Table, in each case, cetylpyridinium chloride greatly enhanced bacterial adhesion to the oil droplets. This phenomenon was also observed microscopically. Enhanced adhesion was not observed for non-cationic surface-active agents: adhesion to all three test oils in the presence of the anionic sodium lauryl sulphate or the nonionic Tween 20, added at the same concentration as the cetylpyridinium chloride (w/v), was 0%. Morever, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 98% when 0.08% cetylpyridinium chloride was present; adhesion of the yeast Candida alpicans rose from 20 to 100% in the presence of 0.16% chlorhexidine digluconate. Adhesion of Acinetobacter calcoaceticus MR-481 rose from 0 to 97% in the presence of 0.18% of cetylpyridinium chloride.

In another experiment, a volunteer swished his mouth with 0.2% saline solution (10 ml) for thirty seconds. To 1 ml of the expectorate were added 0.18 ml of 0.5% cetylpyridinium chloride (water in the case of the control) and 0.44 mm 0.2% saline. Mineral oil 50 (0.2 ml) was then added and the mixtures vortexed for 2 minutes. Following phase separation, the mixtures were examined. Whereas the turbidity of the expectorate was greatly reduced in the presence of cetylpyridinium chloride, presumably due to adsorption of bacteria and debris to the oil droplets, in the absence of this detergent, no increase in turbidity was observed.

The ability of another cationic surface-active agent, chlorhexidine digluconate, to enhance microbial adhesions to oil droplets is shown in the following experiment. Actnetobacter calcoaceticus RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30°C in brain heart infusion broth. The cells were harvested, washed by centrifugation, and suspended in 0.2% saline to a corrected optidal density of ca. 18 at 0 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% chlorhexidine digluconate in water (or water for control) and 0.24 ml water. To each cuvette 0.2 ml of oil was added (vs. control with no oil added) and the mixtures vortexed vigorously for 2 min. After about thirty minutes, the turbidity of the lower aqueous phase was measured at 400 nm and corrected for non-linearity by a standard curve. Adhesion

is calculated as the percent drop in corrected turbidity following the mixing procedure. The results are summarized in the following Table.

Table 2. Adhesion of RAG-1 to various oils in the presence of chlorhexidine digluconate

Additions:	Percent adhesion to			
	n-hexa-decane	isohexa-decane	Sigma* soybean oil	Commercial soybean oil
Chlorhexidine digluconate	95	94	90	89
None	50	66	54	49

* St. Louis, Mo.

As can be seen from the above Table, in each case chlorhexidine digluconate greatly enhances bacterial adhesion to the oil droplets. Moreover, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 98% when 0.1% chlorhexidine digluconate was present.

Adhesion to oil droplets may also be enahnced by polymeric cationic agents, such as chitosan. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 97% when 0.015% chitosan was present; adhesion of the yeast Candida albicans rose from 20 to 95% in the presence of 0.025% chitosan. Adhesion of Acinetobacter calcoaceticus MR-481 rose from 0 to 99% in the presence of 0.019% of chitosan.

The compositions of the invention are suitable for a variety of applications. As a two-phase mouthwash, containing an aqueous phase and an oil phase, the presence of adhesion-enhancing cationic agent (or combination thereof) could remove and bind high levels of oral microorganisms and debris.

The enhanced adhesion of RAG-1 cells to oils in the presence of cationic surfactants similarly teaches the use of cationic surface-active agents to enhance microbial adhesion to oils for commercial purposes, such as immobilization of cells onto oil droplets, or of cells, cell walls, and the like for enhanced adhesion to adjuvant oils in the vaccination field.

In general, salts such as sodium chloride, magnesium chloride and various phosphates, enhance adhesion of microorganisms to surface by countering the net negative charges which generally exist on cell surfaces, and often on substrata surfaces. Weiss et al. include in their U.S. patent 4,525,342, a requirement for salt. However, as stated, it has now been found that the enhancement of microbial adhesion to oils observed in the presence of cetylpyridinium chloride, chlorhexidine gluconate and chitosan is inhibited by sodium chloride and magnesium chloride.

For example, adhesion of C. albicans to hexadecane in the presence of 0.16% cetyl pyridinium chloride fell from 100% to 18% when 0.8% NaCl was present.

In order to obtain a picture as to the efficacy of the compositions of the invention for oral use these were compared with existing mouthwashes as regards their ability to remove a given bacterial film from a solid surface.

There were used a number of commerical products and these were compared with the preparation of the invention defined below as ODEX-35. The following describes and illustrates in detail specifric features of the invention:

Example ODEX-35

Aqueous phase:	
sodium chloride -	0.2% w/w
sodium saccharin -	0.05% w/w
FD&C Blue No. 1 -	0.0001% w/w
cetyl pyridinium chloride -	0.03% w/w
water -	to 100%
Oil phase	
soybean oil -	35.0% w/w
essential oil (mint) -	1.5% w/w

This type of formulation has several advantages. First, the presence of cetylpyridinium chloride results in adsorption of much higher levels of bacterial and oral debris, than when it is absent. Second, the mixture can be presented in a single, regular container, e.g. bottle, obviating the need for a double compartment, double squirt bottle. The user simply shakes for several seconds, the amount of cetylpyridinium chloride is sufficient to provide an emulsion with limited stability which enables the user to pour the desired quantity of mixture and use it directly to swish and gargle. An added advantage of the formulation is that the cetylpyridinium chloride enhances the dye binding capacity of the bacteria and debris so that they are clearly colored blue by the dye, facilitating their observation.

Moreover, the dye and cetylpyridinium chloride may act together in a synergistic fashion as antibacterial agents.

In one experiment, the formulation was mixed, and 10 ml immediately poured into a small cup. Volunteers swished and gargled with the mixture for a thirty second period. Following swishing, the mixture was expectorated into test tubes. It was clearly observed in all cases that the oral bacteria and debris, dyed blue, rose together with the yellow oil droplets, creating a green upper oil:in:water emulsion which was stable for hours. Bacteria, and bacteria-coated epithelial cells were microscopically observed adhering to the oil droplets. The aqueous phase lost its blue color, presumably due to the binding of the dye to the great quantities of bacteria and debris removed onto the oil droplets during the mixing procedure. A drop in the level of bad breath of the volunteers was evident for hours following the swishing procedure. Moreover, volunteers reported a long lasting fresh feeling in their mouths, and did not report any oily taste, despite the high level of oil in the preparation. These observations could not be reproduced if either the oil phase, or the cationic surface-active agent were absent.

It should be emphasized that the compositions of the invention also have a wide range of uses in enhanced desorption of microorganisms for host, industrial and environmental surfaces onto oil droplets. To illustrate this point, we have succeeded in

demonstrating that the above formulation is highly effective in removing adsorbed bacteria from a model surface, i.e. polystyrene, as described in the following experiment.

One ml of a very turbid suspension of RAG-1 cells in 150 mM phosphate buffer was added to a polystyrene cuvette (4 ml capacity). Following 1 minute vortexing, the RAG-1 cells were poured off, leaving a film of adherent cells. The cuvettes were washed briefly with tap water. To each cuvette, 1 ml of mouthwash (when indicated, diluted according to instructions) or water (control) were added and the cuvettes vortexed for 2 minutes. The mouthwash was poured off, 1 ml fresh mouthwash was added, and the cuvettes again vortexed for 2 minutes. The mouthwash was poured of, and the remaining bacterial film stained by adding Gentian Violet to the cuvettes, followed by washing to remove excess stain. The amount of bacterial film was ascertained by measuring the optical absorbance (585 nm) of the dried cuvettes from both sides in a Uvikon spectophotometer. The results are presented in Table 3.

From the following Table it can be seen that among the commercial mouthwashes tested, several (Eludril, P.F. Medicament, 125, rue de la Faisanderie, Paris, France; Tayadent, Taya Ltd., Petach Tiqua, Israel and Act^{R}, Johnson & Johnson, New Brunswick, N.J. 08903) were less able than water to desorb the bacterial film. Menthol Chloraseptic^{R} (Norwich Eaton Pharmaceuticals, Inc., Norwich, N.Y. 13815) and

Veadent^{R} (Glaxo, under licence from Vipont Laboratories Inc., Ft. Collins, U.S.A.)desorbed less than 10% of the adherent bacteria. Cepadont removed 29% of the film. Only ODEX-35 removed the large majority (87%) of the bacterial film. Moreover, ODEX-35 without the oil phase was relatively ineffective (26%) as compared to both phases together (87%).

Table 3

Mouthwash	OD_{585nm} (mean)	Percent removal relative to water control
Control (water	0.300	-
Eludril	0.625	-
Tayadent	0.376	-
Act (green)	0.344	-
Menthol Chloraseptic	0.288	4
Veadent	0.281	6
Cepadont	0.213	29
ODEX-35, aqueous phase only	0.223	26
ODEX-35	0.039	87

In order to further test compositions of the kind described above for use as mouthwashes, several in vivo tests were undertaken. In one of these, various compositions were prepared and swished for two

thirty second periods by a volunteer at bedtime. The following morning, the volunteer swished with ten ml of sterile milk, and 3 ml of the expectorate was mixed in a test tube with 0.12 ml of 0.1% methylene blue. The test tube was allowed to stand at room temperature and the time required for the bottom of the test tube to turn white was observed. It has been previously shown that the time required for a color change from blue to white at the bottom of the test tube correlates highly with microbial counts, thus a table was used to convert the time for color change into relative microbial counts.

It is clear that a positive interaction exists between cetylpyridinium chloride and chlorhexidine. Thus, whereas a sample containing only cetylpyridinium chloride (0.05%) as cationic agent and another sample containing only chlorhexidine digluconate (0.05%) as cationic agent left a high percentage of residual microorganisms in the mouth (over 30%), a combination of 0.05% cetylpyridinium chloride and 0.05% chlorhexidine was highly effective, leaving less than 14% residual microorganisms in one instance, and 11% in another. Moreover, the cetylpyridinium chloride has a better emulsifying ability, whereas chlorhexidine digluconate has better antibacterial activity. Thus, the combination of both together with the oil is highly effective in obtaining a composition which can be effectively emulsified by shaking for a brief period, combined with potent antibacterial ability. Compositions containing from 5-35% (w/w) of soybean oil, cetylpyridinium chloride, and chlorhexidine were highly effective. Another advantage of the two-phase oil:water combination is that moieties which generally require emulsification in regular mouthwashes can be added to the oil phase. For example, in order to introduce peppermint oil into the aqueous phase, it must be solubilized e.g. with Tween. This causes a great reduction in activity of the formula, by inactivating the cations (e.g. chlorhexidine) present.

Thus, when antibacterial cations such as chlorhexidine are present in the aqueous phase, it is preferable to add the oil-soluble components to the oil phase. Such components include flavors, antioxidants (e.g. Vitamin E, BHA, BHTA), and essential oils which have antibacterial activity, e.g. eucalyptus oil, cinnamon oil.

On the other hand, samples in which the essential oil (peppermint) was solubilized in the aqueous phase using Tween 20 were not effective, even though either 0.1% chlorhexidine or a combination of 0.05% of both cetylpyridinium chloride and chlorhexidine were present.

Bad breath is known to be chiefly due to microbial activity in the oral cavity. A sample containing soybean oil, cetylpyridinium chloride and chlorhexidine was tested against a leading commercial mouthwash (Listermint, Warner-Lambert Health Care Ltd., Eastleigh, Hants, England) for its ability to reduce bad breath levels, as determined by oral sulphide levels, using a sulphide monitor, (Interscan Model 1170, Interscan Ltd., Chatsworth, CA). One-quarter inch plastic straws are inserted into the sample inlet and the volunteer allows the end of the straw to enter the oral cavity, and breathes through his nose. The peak and steady-state value are recorded in parts per billion sulphide equivalents. Volunteers swished with either mouthwash for two thirty second periods at bedtime and tested peak and steady-state sulphide levels immediately upon awakening. The inventive sample reduced peak levels by 74%, and steady-state

levels by 70%, as compared to no-mouthwash controls; in contrast, Listermint reduced peak levels by 51% and steady state levels by 45% as compared to no-mouthwash controls. Moreover, in a comparison of the ability of the inventive sample to remove bound microorganisms from a solid surface, in an experiment analogous to that described in Table 3, the sample was superior to a wide variety of commercial mouthwashes in its ability to desorb bound cells.

As an additional example, we have tested formulations whose oil phase contains a combination of olive oil, peppermint oil and eucalyptus oil. One formulation, designated ODEX, has the following composition:

Aqueous phase:	
cetylpyridinium chloride	0.05%(w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.75% (w/w)

Oil phase:	
olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

Another formulation, designated ODEX+ has the following composition:

Aqueous phase:

cetylpyridinium chloride	0.05% (w/w)
chlorhexidine digluconate	0.05% (w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.70% (w/w)

Oil phase:

olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

The ability of these formulations to desorb microorganisms from polystyrene was tested as described in reference to Table 3. ODEX and ODEX+ removed 98% and 92%, respectively, of the bound RAG-1 bacteria.

Moreover, ODEX was tested in its ability to reduce bad breath, as compared to the following controls: (i) a commercial mouthrinse, Cepadont; (ii) a control in which the oil phase was entirely omitted; (iii) a control in which the olive and peppermint oil were omitted, and the eucalyptus oil solubilized in 6% Tween 20; and (iv) no mouthrinsing control. Peak morning odor was measured using the Interscan 1170 sulphide analyzer. Two volunteers rinsed, prior to

bedtime, for two consecutive 30 second periods. Immediately upon awakening, peak sulphide was measured. The results are summed up in Table 5 below:

Table 5: Overnight Effect of ODEX on Bad Breath

FORMULATION TESTED	Parts per billion sulphide equivalents	
	Volunteer 1	Volunteer 2
ODEX	25	20
(i) Cepadont	140	70
(ii) No oil phase	70	40
(iii) Solubilized eucalyptus oil	60	-
(iv) No mouthrinsing	117	150

These data show that the combination of aqueous phase and non-emulsified oil phase containing eucalyptus oil was more effective than any of the controls.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be

considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

CLAIMS

1. A composition for desorbing bacteria from solid surfaces and from living tissues, the composition being in the form of a two-phase preparation which upon shaking forms a temporary oil-in-water emulsion of limited lifetime, said composition comprising:

a) from 59 to 97% w/w of an aqueous phase;

b) from 3 to 50% w/w of a water immiscible oily phase comprising a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydrocarbon or a mixture thereof; and

c) from 0.003 to 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emusion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

2. A composition for desorbing bacteria from solid surfaces and from living tissues according to Claim 1, wherein said amphipatic cationic moiety is selected from surface-active cationic molecules containing a pyridinium moiety, chlorhexidines, monalkyl quaternary ammonium compounds, dialkyl quaternary ammonium compounds, quaterniums and polyquaterniums, amine fluorides, cationic polysaccharides, and cationic polypeptides.

3. A composition for desorbing bacteria from solid surfaces and from living tissues according to Claim 1, wherein said amphipathic

cationic moiety is selected from cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme.

4. A composition according to claim 1, where the oily phase is selected from olive oil, corn oil, coconut oil, soybean oil, safflower oil, octane, decane, tetradecane, hexadecane, white mineral oil or a mixture of any of these.

5. A composition according to claim 1 wherein said amphipathic cationic moiety is a chlorhexidine compound.

6. A composition according to claim 1 wherein said amphipathic cationic moiety is chitosan.

7. A mouthwash according to claim 1 comprising from 0.003 to 0.5 w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

8. A composition according to claim 1 containing up to 0.3% of inorganic salts, such as phosphate, sodium or magnesium chloride, or fluorides.

9. A composition according to claim 1 which contains from 0.005 to 0.5% cetylpyridinium chloride.

10. A composition according to claim 1 which contains from 0.005 to 0.5% cetylpyridinium chloride and from 0.005 to 0.5% of a chlorhexidine compound.

11. A composition according to claim 1 further comprising a pharmacologically acceptable dye which binds to microorganisms and debris in the presence of the other components.

12. A composition for the desorption of bacteria from solids and from tissues in a two-phase form composition which forms a temporary emulsion upon shaking, substantially as hereinbefore described and with reference to the examples.

13. A method for enhancing desorption of microorganisms from host industrial and environmental media onto oil droplets, the method comprising admixing an aqueous suspension of the micro-organisms and oil in the presence of an amphipathic cationic moiety.

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Fig. 1

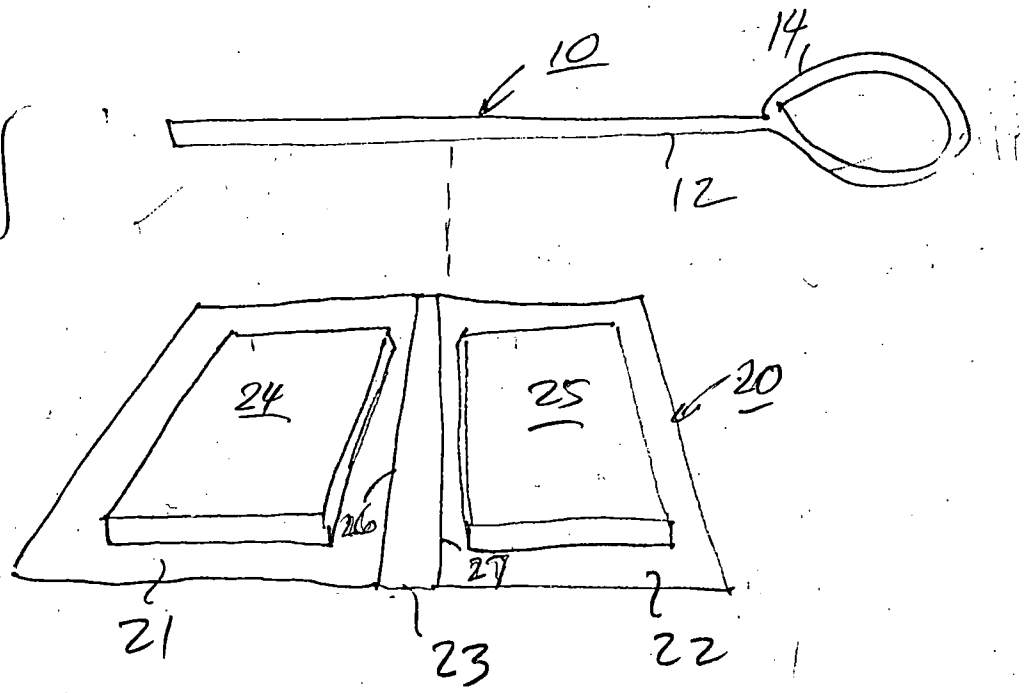
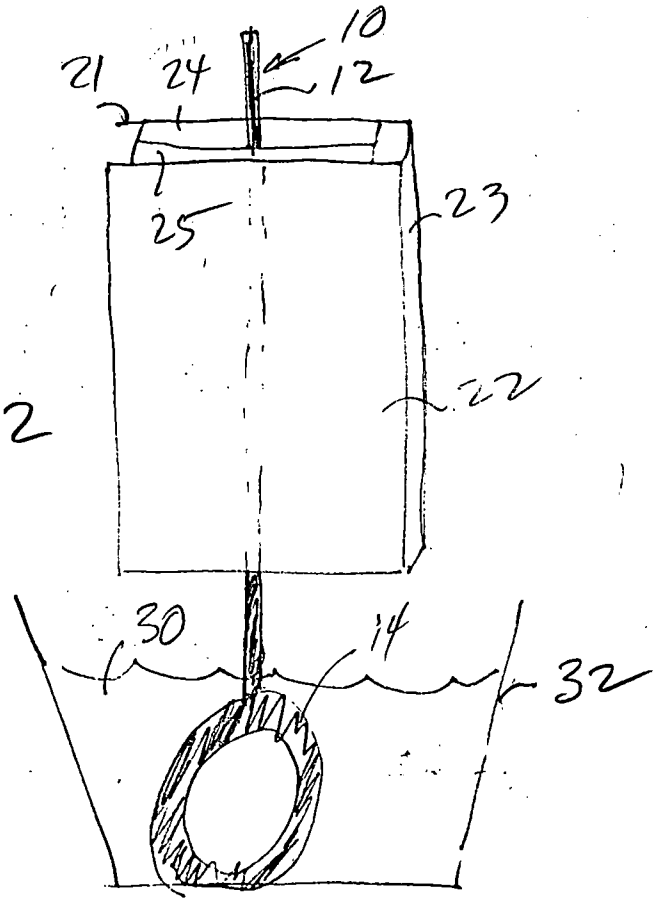


Fig. 2



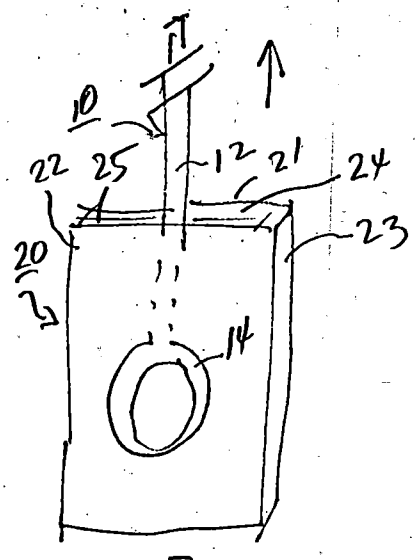


FIG. 3

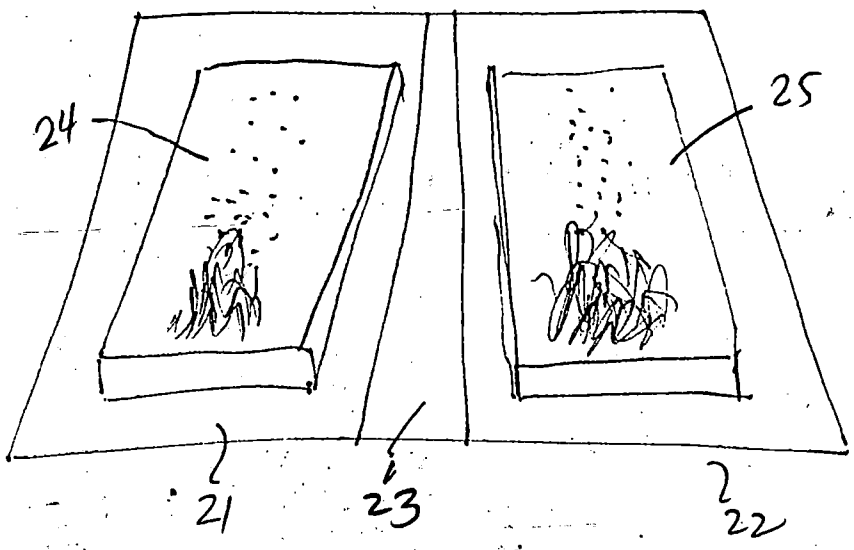


FIG. 4

Fig. 5a

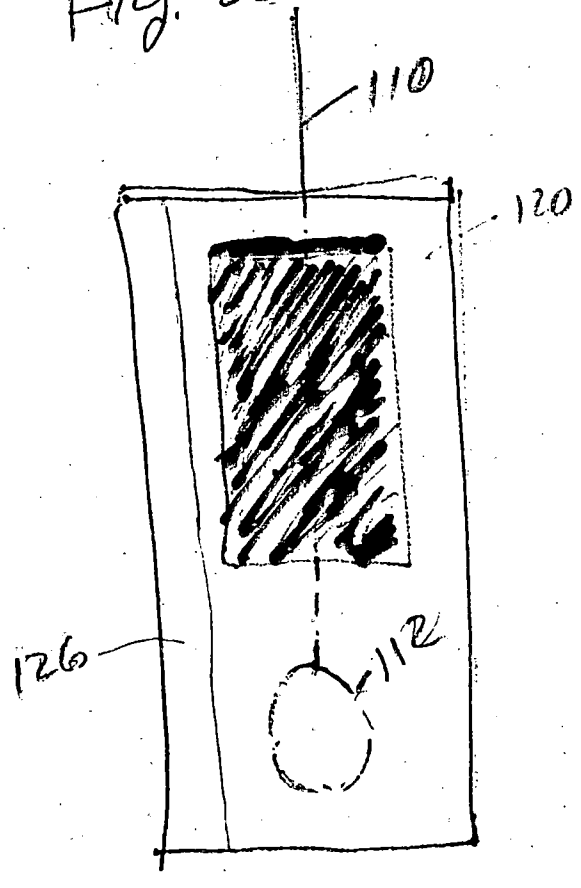
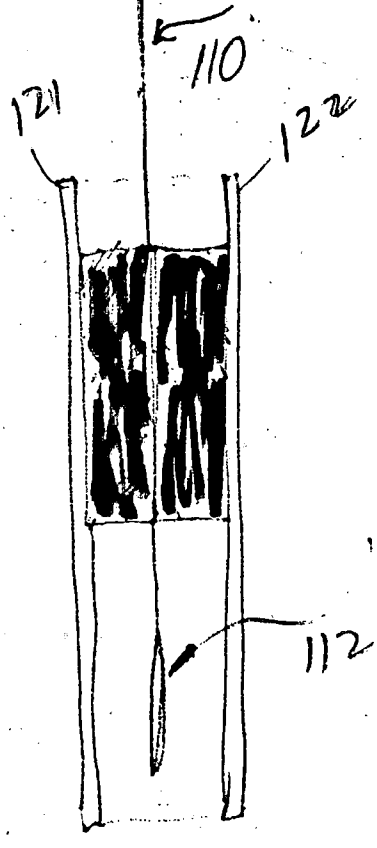


Fig. 5b



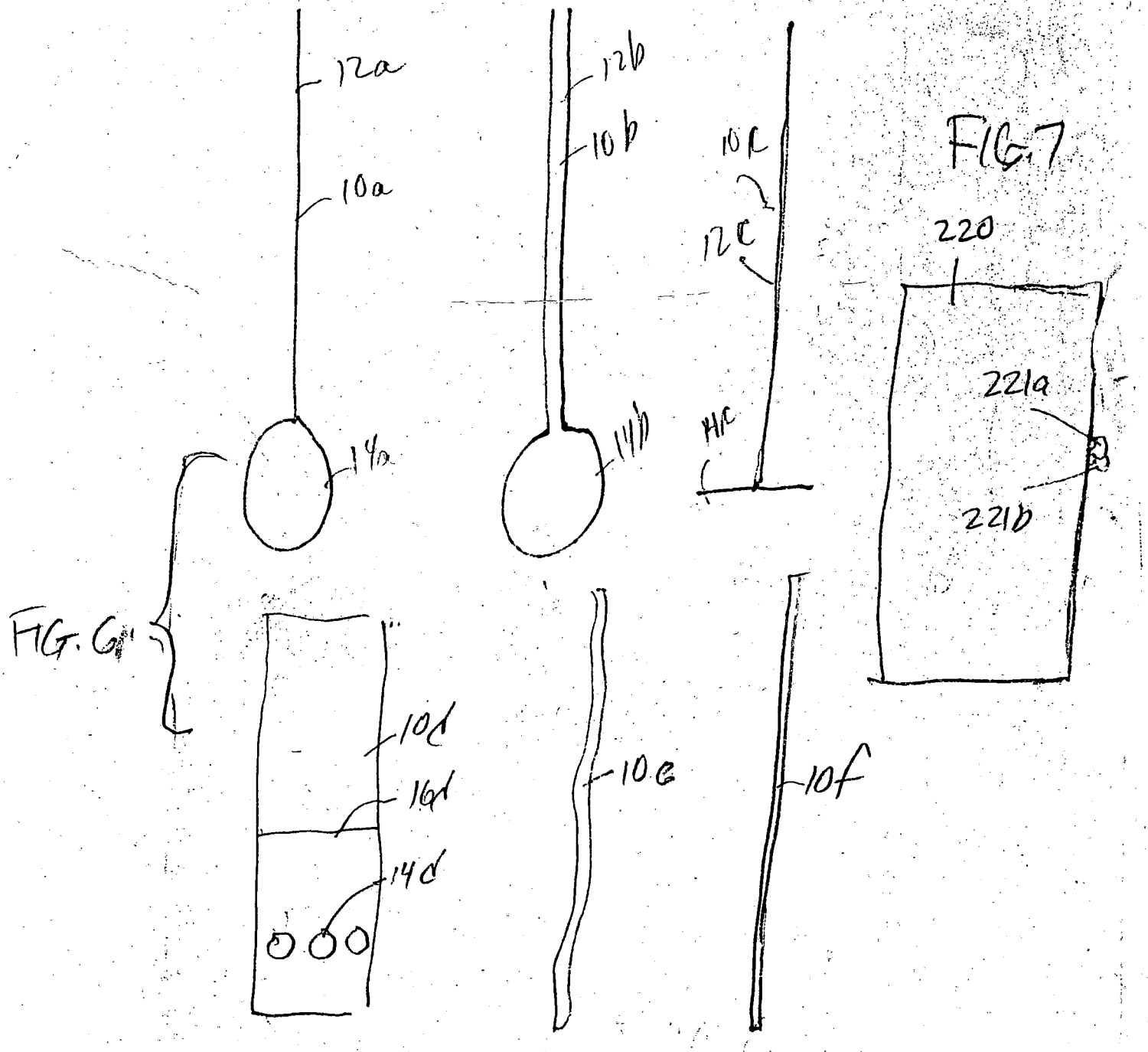

12a
10a
14a
12b
10b
14b
10c
12c
HC
10d
16d
14d
10e
10f

FIG. 6

220
221a
221b

FIG. 7

File: 1989-us-patent-4801547.pdf

United States Patent [19]

Rosenberg

[11] **Patent Number: 4,801,547**

[45] **Date of Patent: Jan. 31, 1989**

[54] **DEVICE FOR DETECTING PRESENCE OF MICRO-ORGANISMS IN A SAMPLE**

[75] Inventor: **Melvyn Rosenberg**, Ramat-Gan, Israel

[73] Assignee: **Ramot University Authority for Applied Research and Industrial Development Ltd.**, Tel Aviv, Israel

[21] Appl. No.: **136,666**

[22] Filed: **Dec. 22, 1987**

[30] **Foreign Application Priority Data**

Jan. 2, 1987 [IL] Israel 81145

[51] **Int. Cl.**[4] **C12M 1/26**

[52] **U.S. Cl.** **435/292**; 435/294; 435/810

[58] **Field of Search** 435/292, 293, 294, 295, 435/296, 299, 300, 301, 810

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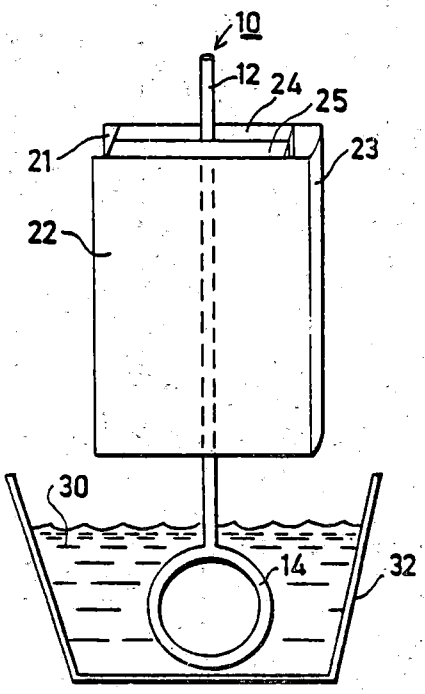
Primary Examiner—James C. Yeung

Attorney, Agent, or Firm—Benjamin J. Barish

[57] **ABSTRACT**

A device for transferring a portion of a sample to a culture medium to detect the presence of microorganisms in the sample comprises: a supporting member including two panels hinged together along one edge to permit the panels to assume a folded condition with one panel folded over the other, or an open condition; a culture medium carried on the face of one or both panels facing each other when the panels are in their folded condition; and a transfer member adapted to be supported between the faces of the two panels when in their folded condition and dimensioned such that the opposite ends of the transfer member project from the opposite ends of the culture medium, whereby one projecting end may be brought into contact with the sample to pick-up a part thereof, and the opposite projecting end may be grasped by the user and pulled to move the one end of the transfer member between the contacting faces of the folded panels to transfer and dilute, by smearing, the picked-up sample to the culture medium.

19 Claims, 4 Drawing Sheets



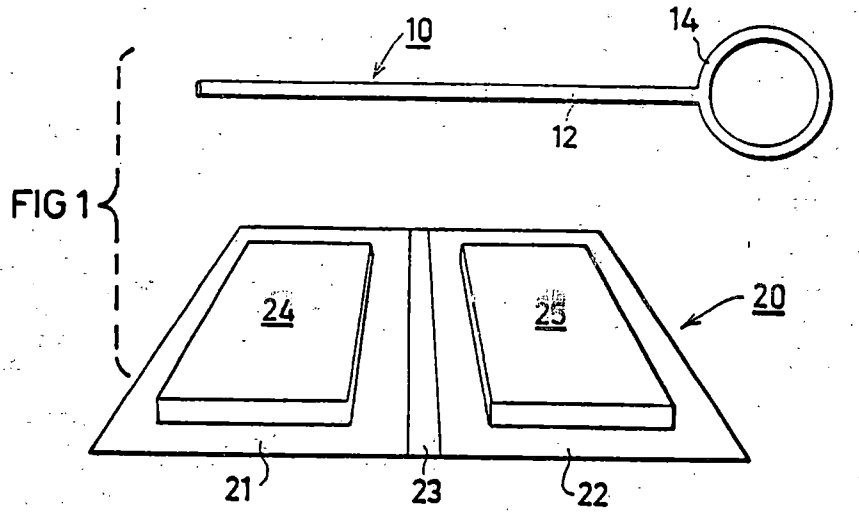

10
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FIG 1
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22

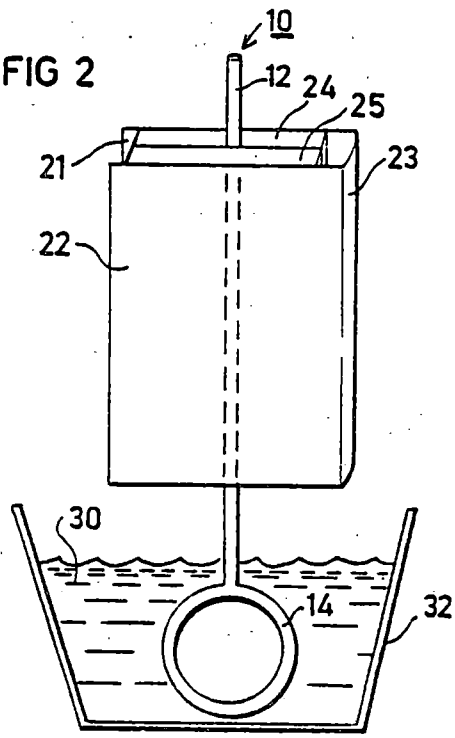
FIG 2
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12
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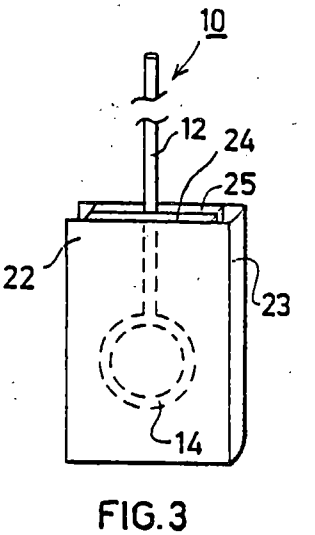
10
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FIG. 3

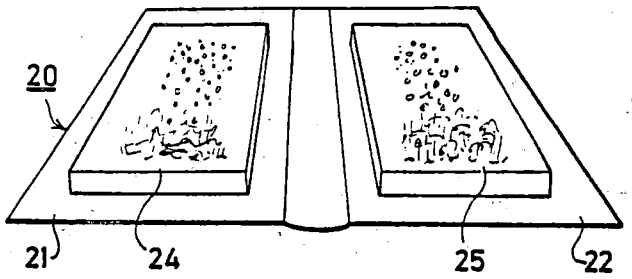
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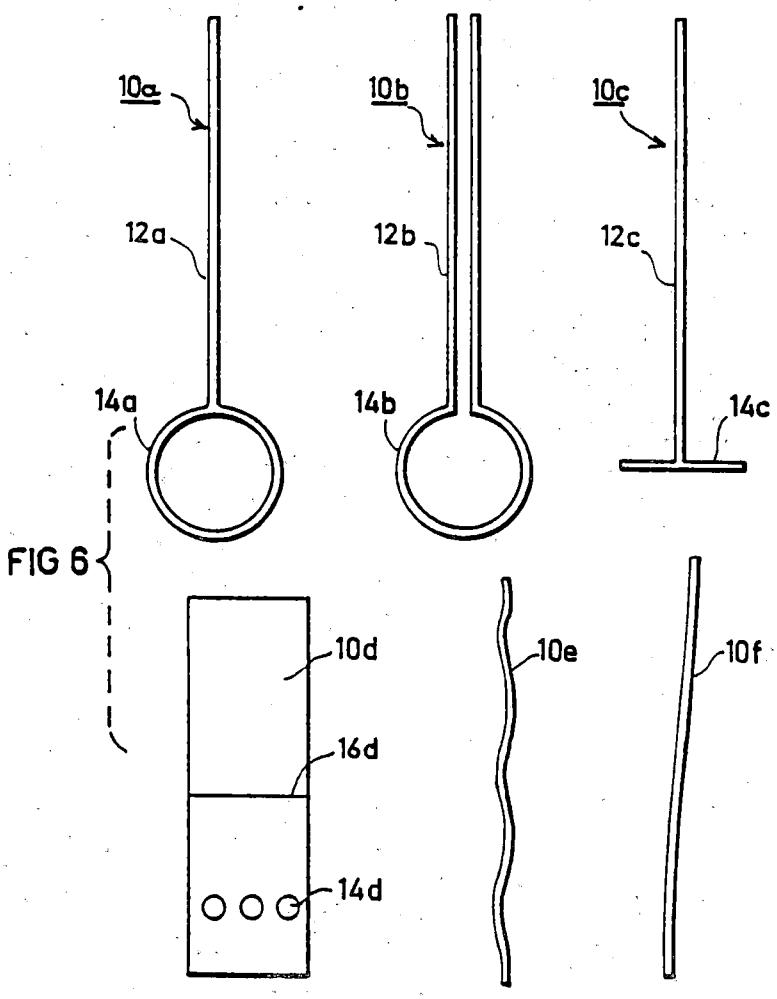
FIG 4


10a
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14b
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14c
10d
16d
14d
10e
10f

FIG 6

FIG 5a

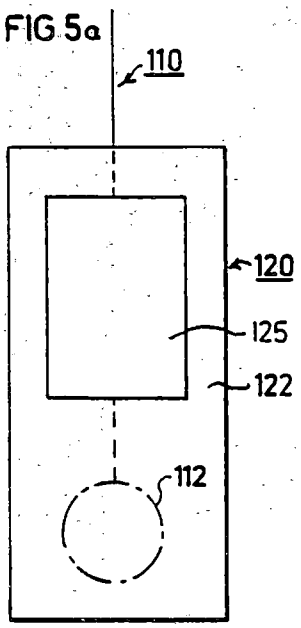


FIG 5b

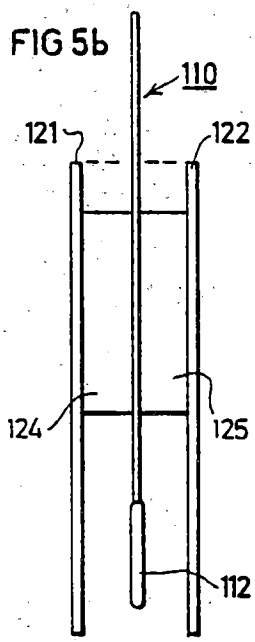


FIG 7

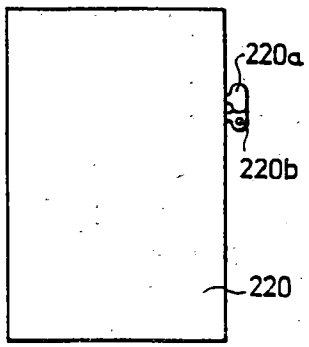


FIG. 8a

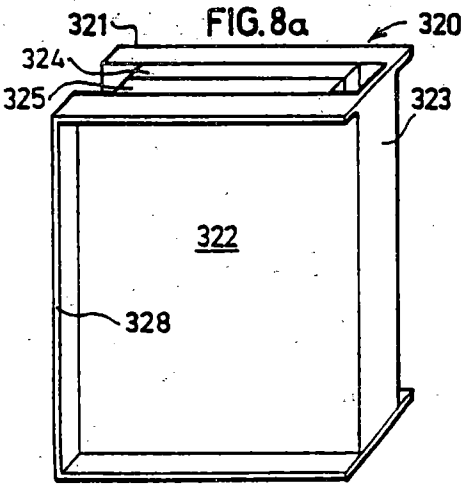


FIG 8b

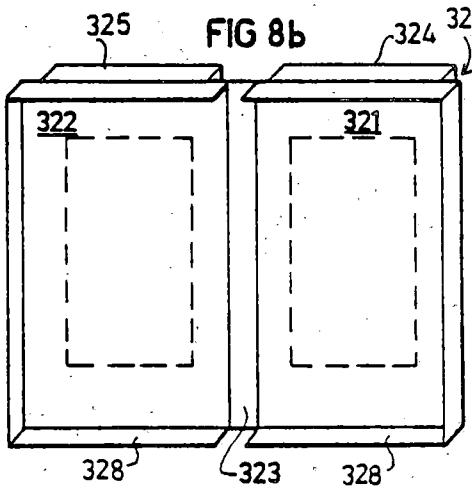
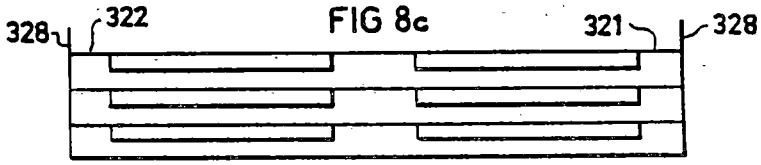


FIG 8c



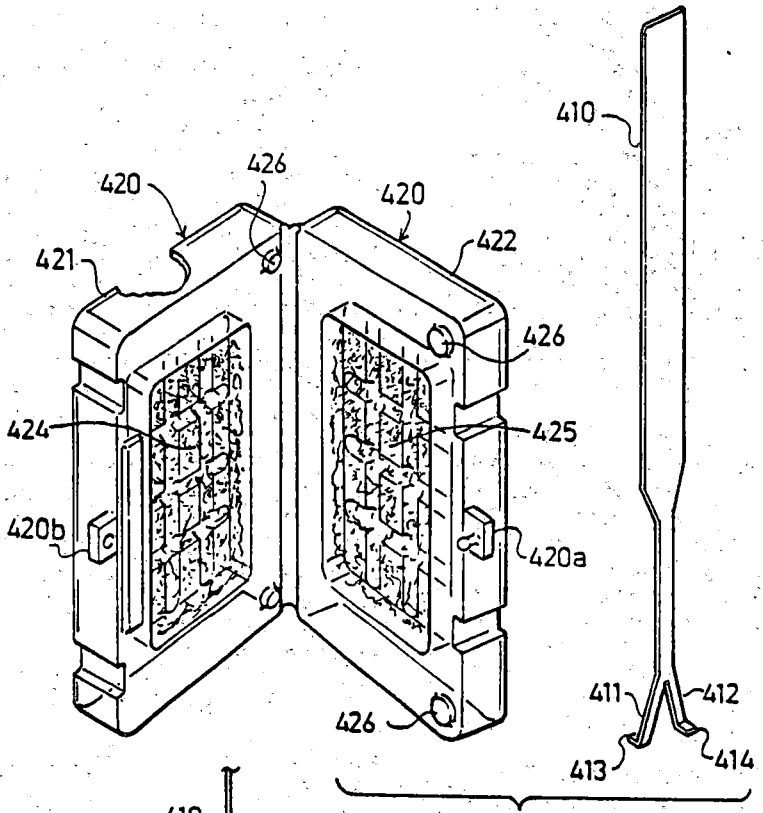

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FIG 9a

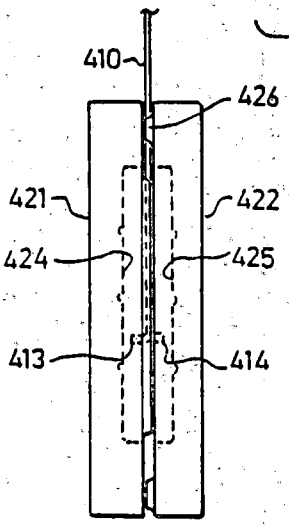
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FIG 9b

DEVICE FOR DETECTING PRESENCE OF MICRO-ORGANISMS IN A SAMPLE

BACKGROUND OF THE INVENTION

The present invention relates to devices for detecting the presence of micro-organisms in a sample, and particularly to devices for transfering a portion of the sample to a culture medium in order to detect the presence and extent of microbial contamination thereof.

A present method for detecting the presence and extent of microbial contamination of liquids, for example in a urine sample, is by the use of a paddle or dipslide which contains a solid medium on one or both sides. The paddle or dip slide is dipped into the liquid to be sampled, and then is removed, and incubated for microbial growth.

One of the major drawbacks of this method is that individual colonies can only be obtained within a very narrow range of microbial concentrations. Obtaining individual colonies is essential for determining the general identity of each contaminating species, whether there is one or more types of contaminating bacteria. When commercial dipslides and paddles are used for detecting micro-organisms in urine samples, for example, positive urine cultures frequently yield confluent growth, which is difficult to observe, study and transfer for further investigation.

BRIEF SUMMARY OF THE INVENTION

An object of the present invention is to provide a novel device for transferring a portion of a sample to a culture medium which device has advantages in the above respects when used to detect the presence of micro-organisms in the sample.

According to the present invention, there is provided a device for transferring a portion of a sample to a culture medium to detect the presence of microorganisms in the sample, comprising: a supporting member including two panels hinged together along one edge to permit the panels to assume a folded condition with one panel folded over the other, or an open condition; a culture medium carried on the face of at least one of the panels facing the other panel when the panels are in their folded condition; and a transfer member adapted to be supported between the faces of the two panels when in their folded condition and dimensioned such that the opposite ends of the transfer member project from the opposite ends of the culture medium, whereby one projecting end may be brought into contact with the sample to pick-up a part thereof, and the opposite projecting end may be grasped by the user and pulled to move the one end of the transfer member between the contacting faces of the folded panels to transfer, by smearing, the picked-up sample to the culture medium.

It will thus be seen that a device constructed in accordance with the present invention provides a simple and convenient means for transfer and dilution of the sample on the culture medium surface thereby enabling colony isolation, irrespective of the microbial concentration of the sample. After the transfer of the sample to the culture medium, the two panels may be opened to a side-by-side relationship, thereby producing a convenient and compact arrangement for incubation, or otherwise processing, a plurality of the devices at the same time.

In the preferred embodiments of the invention described below, the culture medium is carried on both faces of the two panels facing each other when the panels are in their folded condition, whereby the transfer member transfers a part of the picked-up sample to the culture media on the faces of both the panels. The faces of both panels may carry the same culture medium; alternatively, they may carry different culture media for cultivating different kind of micro-organisms.

The supporting member may be of sheet material, such as plastic, folded along one edge to form the hinge integrally with the panels. According to another arrangement, the supporting member may include two separate panels joined along the one edge by a flexible strip. In all the disclosed arrangements, the two panels may also include a retainer for releasably retaining them in their folded condition.

Further features and advantages of the invention will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. **1** illustrates the main components of one form of device constructed in accordance with the present invention for transferring a portion of a sample to a culture medium to detect the presence of micro-organisms in the sample;

FIG. **2** illustrates the device of FIG. **1** when used for transferring a portion of the sample to a culture medium;

FIG. **3** illustrates the device of FIG. **2** during the transferring operation;

FIG. **4** illustrates the supporting member and the culture medium after transfer and incubation of the sample thereto;

FIGS. **5***a* and **5***b* are side and bottom views, respectively, illustrating another construction of device for transferring samples to culture media;

FIG. **6** illustrates a number of different types of transfer members which may be used with the device of the preceding figures;

FIG. **7** illustrates a modification wherein the supporting member for the culture media is provided with retainers for releasably retaining the two panels in their folded condition;

FIGS. **8***a*–**8***c* illustrate a further modification wherein the supporting members are provided with spacer means enabling a plurality of them to be stacked, FIGS. **8***a*, **8***b*, **8***c*, illustrating the device in it closed, open and stacked condition, respectively; and

FIGS. **9***a* and **9***b* illustrate a further modification, wherein the culture medium is recessed in the confronting faces of the two panels, FIG. **9***a* being a perspective view of both the supporting member and transfer member, and FIG. **9***b* being a side view illustrating the manner of using the transfer device.

DESCRIPTION OF PREFERRED EMBODIMENTS

The main components of the transfer device illustrated in FIG. **1** are: a transfer member, generally designated **10**, for picking-up a portion of the sample; and a supporting member, generally designated **20**, carrying the culture medium to which a part of the sample is to be transferred.

The transfer member **10** illustrated in FIG. **1** includes a flattened handle **12**, and a flattened loop **14** at one end for picking up a portion of the sample. The supporting

member 20 includes two panels 21, 22 hinged along one edge, as shown at 23, to permit the panels to assume either an open condition as illustrated in FIG. 1, with the two panels in side-by-side relationship, or a folded condition, as illustrated in FIG. 2, with one panel folded over the other. A layer of culture medium 24, 25, is carried on the face of each panel 21, 22 facing the other when the panels are in their folded condition.

As shown particularly in FIG. 2, the transfer loop 10 is adapted to be supported between the faces of the two panels 21, 22 when in their folded condition. In addition, the transfer member illustrated in FIGS. 1 and 2 is dimensioned such that its opposite ends project from the opposite ends of the two panels 21, 22. Thus, the loop 14 projecting from one end of the two panels 21, 22 may be dipped into the sample 30 contained within a container 32; the opposite projecting end of the transfer member, namely the opposite end of handle 12, may be grasped by the user and pulled to move loop 14 between the contacting faces of the folded panels 21, 22 to transfer and dilute, by smearing, the picked up sample to the culture media 24 and 25.

FIG. 2 illustrates the transfer member 10 in its initial condition wherein its loop 14 is dipped within the sample 30; and FIG. 3 illustrates the transfer member being pulled upwardly so as to smear and thus dilute the picked-up sample along the contacting faces of the two layers of culture media 24, 25. The loop 14 is moved for the complete length of the two supporting panels 21, 22 so that when the transfer member is removed from between them, the sample picked-up by loop 14 will have been smeared across the complete lengths of the two culture medium layers 24, 25. The two panels 21, 22 may then be opened, as shown in FIG. 4, and the supporting member may then be incubated or otherwise processed for culturing the micro-organisms deposited on the culture medium layers 24, 25, in order to identify the micro-organisms originally present in the sample.

The culture medium of layer 24 may be the same or different from the culture medium of layer 25. A different culture medium may be used in order to identify different kinds of micro-organisms in the sample.

FIGS. 5*a* and 5*b* illustrate a modification wherein the pick-up end of the transfer member 110 does not project from the two panels 121, 122 of the supporting member 120 in the folded condition of the two panels: rather, it is enclosed between the two panels in their folded condition. Thus, the culture medium layers 124 and 125 of the two panels 121 and 122, respectively, are sufficiently thick, and cover only the upper end of the two supporting panels, in order to provide spaces between, the opposite faces of the transfer member 110 and the panels and their culture medium layers 124, 125. In addition, the transfer member 110 is dimensioned so that its pick-up loop 112 does not project through the end of the supporting panels 121, 122, but rather is enclosed by the lower ends of the two supporting panels and spaced from their confronting faces by the culture media layers 124, 125. Such an arrangement provides some protection against contamination of the loop 112 before it is dipped into the sample, while at the same time permits the loop to pick-up a portion of the sample when dipped into it. Alternatively, the loop may be pushed out from between the supporting panels at the time the loop is to be dipped into the sample.

Pulling the transfer member 110 between the two panels 121, 122 of the supporting member 120 will effectively transfer, by smearing, the picked-up sample to the two culture medium layers 124, 125 in the same manner as described above, after which the two supporting panels may be opened and incubated, or otherwise treated, in order to identify the micro-organisms.

FIG. 6, illustrates a number of examples of transfer members that may be used. Thus, transfer member 10*a* is of the loop-type including a handle 12 and a flattened loop 14; transfer member 10*b* is similar to that of 10*a* except that the handle 12*b* is constituted of the two ends of a wire bent at its mid portion to form the loop 14*b*. Transfer member 10*c* includes a straight-wire handle 12*c* and a cross-bar 14*c* for picking up the sample.

Transfer member 10*d* is of sheet material and is formed at its lower end with one or more openings or perforations 14*d* for picking-up a part of the sample when dipped therein. As one example, transfer member 10*d* may be of plastic sheet material; and the openings 14*d* may be calibrated to accept a predetermined volume of the sample, particularly when the transfer member is dipped into the sample to the level indicated by line 16*d*. Alternatively, transfer member 14*d* may be of paper or wood calibrated so as to absorb a predetermined volume of the sample when the transfer member is dipped to the level of line 16*d*.

It will be appreciated that the loops 14*a* or 14*b* of transfer members 10*a* and 10*b* may be similarly precalibrated to accept a predetermined volume of the sample.

FIG. 6 illustrates two additional forms of transfer members that may be used, namely transfer member 10*e* in the form of a string, and transfer member 10*f* in the form of a wire. It will be appreciated that still other forms of transfer members may be used.

FIG. 7 illustrates a further variation wherein the two panels of the supporting member, therein designated 220, are provided with retainers to releasably retain the two panels in their folded condition, preferably with the transfer member (not shown) in between. The retainers for this purpose may be in the form of projections 220*a* 220*b* which engage each other when the two panels are closed in their folded condition as illustrated, and which are yieldable sufficiently so as to permit disengagement of the two projections by merely pressing them toward each other, similar to conventional arrangements used in plastic containers for releasably retaining the container lid closed.

FIGS. 8*a*–8*c* illustrate further modifications wherein the device includes spacer means enabling a plurality of them to be stacked for sterilization, incubation, storage or other purposes. The device illustrated in these figures, and therein generally designated 320, is basically of the same construction as described above with respect to FIGS. 1 and 2, including two panels 321, 322 interconnected by a hand 323 with each panel carrying a layer of a culture medium 324 and 325, respectively. In this modification, however, an upstanding rim 328 is formed along the outer edge of each of the panels 321, 322. Rim 328 may be, for example, in the form of a flange, continuous rib, or discontinuous rib, formed along the outer edge of each of the panels 321, 322 on the face of the panel opposite to that carrying the culture medium 324, 325. The height of each such rim 328 should be slightly greater than the thickness of the culture media layers 324, 325, so that when a plurality of such devices, in their open condition as shown in FIG. 8*c*, are stacked one on top of the other, the culture medium 324 of one device will be spaced from the opposite face of the next adjacent device.

In all the described embodiments, the supporting member for supporting the two culture medium layers may be of sheet material e.g., plastic, folded along one edge to form the hinge integral with the panels. This is shown particularly in FIG. 1, wherein supporting panel 20 is constituted of a single plastic sheet folded along the two lines 26, 27 to define the hinge 23 integral with the two panels 21, 22. An alternative arrangement is illustrated in FIG. 5*a*, wherein the supporting member 120 includes two separate panels 121, 122 joined along one edge by a flexible plastic strip 126 to define the hinge permitting the two panels to be moved either to their open or folded positions.

Experiments have been performed using flat strips of paper, parafilm, plastic material, and various flat metal loops. The flat strips were dipped into urine seeded with *Escherichia coli* alone, or together with *Serratia marcescens*. Agar surfaces were produced by pouring autoclaved agar-containing bacteriological media (brain heart infusion and MacConkey) onto glass slides or petri dishes, and allowing to cool. Each strip or loop was then placed between two agar surfaces and dipped into the liquid sample. The strip was then pulled through the contacting agar surfaces. The agar surfaces were then separated and incubated for microbial growth at 30° C. Results showed that this procedure succeeded in diluting extremely concentrated bacterial suspensions, and provided individual colonies, whereas dipping commercial paddles into the same suspensions resulted in confluent growth.

FIGS. 9*a* and 9*b* illustrate a further modification in the transfer device, including a transfer member 410 and a supporting member 420 formed with two hinged panels 421, 422 whose confronting faces are each provided with a layer of culture medium 424, 425. In this case, however, the confronting faces of the two panels 421, 422 are recessed. In addition, panel 422 is provided with circular projections 426 at each of its outer corners. The arrangement is such that when the two panels are closed, as shown in FIG. 9*b*, the two layers of culture media 424, 425 do not actually contact each other.

The two panels may be releasably retained in their closed, folded condition, by a pair of retainer projections 420*a*, 420*b*, similar to the arrangement illustrated in FIG. 7.

The transfer member 410 is in the form of a flat strip of stiff plastic material formed with a pair of arms 411, 412 joined in a Y-formation so as to provide a pair of spaced tips 413, 414 at their outer ends, constituting the pick-up end of the transfer member. Tip 413 is bent in one direction out of the plane of the strip, and tip 414 is bent in the opposite direction, such that the free edge of each tip contacts one culture medium and the juncture edge (juncture with the hand-grasped portion) contacts the other culture medium (FIG. 9*b*). Thus, after incubation there is produced two streaks of different dilution on each culture medium.

The transfer device illustrated in FIGS. 9*a* and 9*b* provides the additional advantages that, when the two panels are closed, their respective culture media do not contact each other, and therefore cannot bleed into each other; and that, after the smearing operation, it is not necessary to open the folded panels before incubation.

While the invention has been described with respect to several preferred embodiments, it will be appreciated that many other variations, modifications and applications of the invention may be made.

What is claimed is:

1. A device for transferring a portion of a sample to a culture medium to detect the presence of microorganisms in the sample, comprising:

a supporting member including two panels hinged together along one edge to permit the panels to assume a folded condition with one panel folded over the other, or an open condition;

a culture medium carried on the face of at least one of said panels facing the other panel when the panels are in their folded condition;

and a transfer member adapted to be supported between the faces of the two panels when in their folded condition and dimensioned such that the opposite ends of the transfer member project from the opposite ends of the culture medium, whereby one projecting end may be brought into contact with the sample to pick-up a part thereof, and the opposite projecting end may be grasped by the user and pulled to move said one end of the transfer member between the contacting faces of the folded panels to transfer and dilute, by smearing, the picked-up sample to said culture medium.

2. The device according to claim 1, wherein a culture medium is carried on both faces of the two panels facing each other when the panels are in their folded condition, whereby said transfer member transfers a part of the picked-up sample to the culture media on the faces of both said panels.

3. The device according to claim 1, wherein said supporting member is of sheet material folded along said one edge to form a hinge integral with said panels.

4. The device according to claim 3, wherein said sheet material is plastic.

5. The device according to claim 1, wherein said supporting member includes two separate panels joined along said one edge by a flexible strip.

6. The device according to claim 1, wherein said two panels include a retainer for releasably retaining them in their folded condition.

7. The device according to claim 1, wherein said transfer member is calibrated to accept a predetermined volume of the sample.

8. The device according to claim 1, wherein the supporting member is formed with spacer means enabling a plurality of the supporting member to be stacked with their culture media spaced from adjacent supporting members.

9. The device according to claim 2, wherein each culture medium is carried in a recess in its respective panel such that the two culture media do not directly contact each other in the folded condition of the panels.

10. The device according to claim 9, wherein the transfer member is a flat strip formed with a pair of spaced tips at its pick-up end, which tips are bent in opposite directions such that each directly contacts one of the culture media during use of the transfer member.

11. A device for transferring a portion of a sample to a culture medium to detect the presence of microorganisms in the sample, comprising:

a supporting member including two panels hinged together along one edge to permit the panels to assume a folded condition with one panel folded over the other, or an open condition;

a culture medium carried on the face of each of said panels facing the other panel when the panels are in their folded condition;

and a transfer member adapted to be supported between the faces of the two panels when in their folded condition and dimensioned such that the opposite ends of the transfer member project from the opposite ends of the culture media, whereby one projecting end may be brought into contact with the sample to pick-up a part thereof, and the opposite projecting end may be grasped by the user and pulled to move said one end of the transfer member between the contacting faces of the folded panels to transfer and dilute, by smearing, the picked-up sample to said culture media.

12. The device according to claim 11, wherein said supporting member is of sheet material folded along said one edge to form a hinge integral with said panels.

13. The device according to claim 12, wherein said sheet material is plastic.

14. The device according to claim 11, wherein said supporting member includes two separate panels joined along said one edge by a flexible strip.

15. The device according to claim 11, wherein said two panels include a retainer for releasably retaining them in their folded condition.

16. The device according to claim 11, wherein said transfer member is calibrated to accept a predetermined volume of the sample.

17. The device according to claim 11, wherein the supporting member is formed with spacer means enabling a plurality of the supporting member to be stacked with their culture media spaced from adjacent supporting members.

18. The device according to claim 11, wherein each culture medium is carried in a recess in its respective panel such that the two culture media do not directly contact each other in the folded condition of the panels.

19. The device according to claim 18, wherein the transfer member is a flat strip formed with a pair of spaced tips at its pick-up end, which tips are bent in opposite directions such that each directly contacts one of the culture media during use of the transfer member.

* * * * *

File: 1989-us-patent-4873317.pdf

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Arnold J. Quigg

Commissioner of Patents and Trademarks

Attest Melvinia Gary

NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number, and timing of the maintenance fees required may be changed by law or regulation.

United States Patent [19]

Rosenberg et al.

[11] **Patent Number: 4,873,317**

[45] **Date of Patent: Oct. 10, 1989**

[54] **METHOD OF SEPARATING OR PURIFYING PROTEINS AND OTHER BIOPOLYMERS**

[76] Inventors: **Melvyn Rosenberg,** 12 Yeda Am Street; **Ilana Eli,** 22 Shazar Street, both of Ramat-Gan, Israel

[21] Appl. No.: **22,225**

[22] Filed: **Mar. 5, 1987**

[30] **Foreign Application Priority Data**

Mar. 13, 1986 [IL] Israel 78130

[51] **Int. Cl.**[4] **C07K 3/12;** C07K 3/20; C07K 3/24; C12P 21/00

[52] **U.S. Cl.** **530/412;** 530/412; 530/413; 530/414; 530/415; 530/416; 530/417; 530/418; 530/422; 530/423; 530/424; 530/425; 530/426; 435/68; 435/99; 435/101

[58] **Field of Search** 530/412-418, 530/422-426; 435/68, 101, 99

[56] **References Cited**

U.S. PATENT DOCUMENTS

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Primary Examiner—Delbert R. Phillips
Assistant Examiner—Abdel A. Mohamed
Attorney, Agent, or Firm—Benjamin J. Barish

[57] **ABSTRACT**

A method for the separation or purification of biopolymers comprises adsorbing the biopolymer on the surface of liquid oil droplets; and separating the adsorbed biopolymer from the oil droplets. The adsorbed biopolymer is separated from the oil droplets by mixing the droplets in an aqueous liquid, removing the lower aqueous phase and adding a fresh aqueous phase to the droplets, cooling the mixture to solidify and coalesce the oil and to cause it to release the adsorbed biopolymer to the fresh liquid, separating the fresh liquid and the biopolymer from the coalesced oil, separating the biopolymer from the fresh liquid.

16 Claims, 1 Drawing Sheet

FIG1

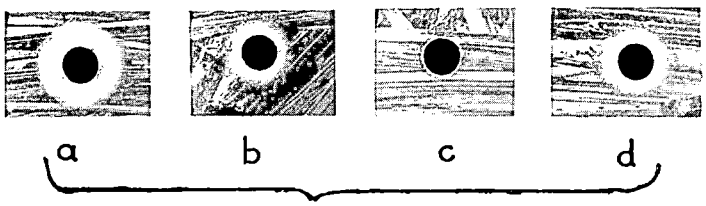

FIG 2

METHOD OF SEPARATING OR PURIFYING PROTEINS AND OTHER BIOPOLYMERS

BACKGROUND OF THE INVENTION

The present invention relates to a method for the separation or purification of proteins and other biopolymers. The invention is believed to be particularly applicable for the separation or purification of proteins such as interleukin-2, insulin, α- and γ-interferon, antibodies, growth hormones, and various enzymes.

The separation or purification of proteins and other biopolymers on the basis of their relative hydrophobic properties is of considerable basic and applied interest. The main techniques currently employed are based on chromatography utilizing solid hydrophobic phases such as n-alkyl and aryl agaroses, and n-alkyl-bonded silica supports. Following adsorption of the amphipathic moieties to the solid support, elution may be carried out by decreasing salt gradients, or increasing the concentrations of detergents or organic solvents. Problems which may be encountered with such an approach include denaturation, elution and the expense of processing large quantities of material.

The present invention relates to an alternative procedure for the separation of proteins and other biopolymers.

BRIEF SUMMARY OF THE INVENTION

According to the present invention, there is provided a method for the separation of biopolymers comprising the steps of adsorbing the amphipathic biopolymer on the surface of liquid oil droplets; and separating the adsorbed biopolymer from the liquid oil droplets.

In the preferred embodiments of the invention described below, the adsorbed bipolymer is absorbed on the surface of the oil droplets by mixing the droplets in an aqueous liquid; separation is achieved by removing the lower aqueous phase and adding a fresh aqueous phase to the droplets, cooling the mixture to solidify and coalesce the oil and to cause it to release the adsorbed biopolymer to the fresh liquid, separating the fresh liquid and the biopolymer from the coalesced oil, and separating the biopolymer from the fresh liquid. The oil droplets are preferably of liquid hexadecane, but it will be appreciated that other oil droplets could be used, for example, octadecane or vegetable oils, e.g., coconut oil, preferably having melting points of −20° C. to 45° C., or silicone-based oils, or fluorocarbons, or other oils.

Further features and advantages of the invention will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is described below with respect to several examples and the accompanying tables and drawings; in the drawings:

FIG. 1 illustrates the results produced in the SDS-Polyacrylamide gel electrophoresis of the seven marker proteins mixed in the presence of hexadecane; and FIG. 2 illustrates the results produced in the adsorption and desorption of lysozyme from clarified human saliva, the lysozyme activity having been assayed on lawns of *Micrococcus lysodeikticus.*

DESCRIPTION OF PREFERRED EMBODIMENTS

EXAMPLES

Following are several examples of the invention:

EXAMPLE 1—Extraction of a Commercial Protein Mixture

The commercial proteins mixture used was the Dalton Mark VII-L (Sigma, St. Louis, MO), a lyophilized mixture of seven proteins, as follows:

1. bovine albumin, approx. 66 Kd;
2. ovalbumin, approx. 45 kD;
3. glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle, approx. 36 kD (subunit);
4. carbonic anhydrase from bovine erythrocytes, approx. 29 kD;
5. treated trypsinogen from bovine pancreas, approx. 24 kD;
6. trypsin inhibitor from soybean, approx. 20.1 kD;
7. α-lactalbumin, approx. 14.2 kD.

The mixture was dissolved in distilled water to a final concentration of 1 mg/ml protein. To one ml of aqueous protein suspension in an acid-washed, round bottom test tube, 0.5 ml hexadecane was added, and the mixture vortexed for 2 min at room temperature. Following phase separation, the lower aqueous phase was removed; one ml of distilled water was added to the test tube, and the contents incubated at 4 C.

Following freezing of the hexadecane layer, the test tube was allowed to equilibrate with room temperature and the lower aqueous phase again removed. Aliquots or the lower aqueous phase prior to and following the hexadecane freezing and thawing procedure were run on a 15% SDS-polycrylamide gel, as described in U. K. Lamemmli, Nature, 227, 1 (1971).

EXAMPLE 2—Extraction of Lysozyme from Human Saliva

Unstimulated, whole saliva was collected from one donor, and clarified by centrifugation at 12,000 g for 10 min. To 1 ml of clarified saliva was added 0.5 ml of hexadecane and the mixture vortexed for 2 min at room temperature. Following phase separation, the lower aqueous phase was removed. The hexadecane layer was washed with 1.0 ml PBS (phosphate buffered saline) including:

1. 8 g NaCl,
2. 0.2 g KCl,
3. 1.15 g Na_2HPO_4,
4. 0.2 g KH_2PO_4 and,
5. distilled water to one liter, pH 7.2.

The wash saline was subsequently removed and replaced with 1.0 fresh PBS.

Contents of the test tube were incubated at 4 C. so to solidify the hexadecane layer; the test tube was then allowed to equilibrate with room temperature, with concomitant melting of the hexadecane.

The foregoing is summarized in the following Table 1:

TABLE 1

Sample	Relative Activity
Untreated clarified saliva	1.00
Following mixing with hexadecane	0.30
Activity adsorbed to interface following mixing procedure	0.70
Aqueous sample following solidification and	0.30

TABLE 1-continued

Sample	Relative Activity
melting of hexadecane layer	

In order to assay lysozyme activity, 25 μl aliquots were applied in quadruplicate to paper pads (Schleicher and Schull, Dassel, West Germany, No. 321260). Following drying, the pads were placed on lawns of Micrococcus lysodeikticus (ATCC 4698) spread on nutrient agar plates. Following 18 h incubation at 30 C. the diameters of the inhibition zones were compared with those obtained using known salivary dilutions and the relative activity obtained by interpolation.

EXAMPLE 3—Extraction of Lysozyme from PBS

Lysozyme (Sigma, St. Louis, MO, No. L6876, from chicken egg white) was dissolved in 1 ml PBS to a final concentration of 200 g/ml and used fresh. To one ml of solution was added 0.5 ml hexadecane, and the mixture vortexed three times for two minute durations.

The lower phase was immediately removed and 1 ml of distilled water was added to the hexadecane. Desorption was carried out as described above. Twenty-five μl aliquots of the liquid removed prior to and following the solidification and melting procedure were assayed for enzyme activity as described in Example 1. Relative activity was calculated from two calibration curves obtained using various lysozyme concentrations, dissolved in water or PBS, respectively.

The foregoing is summarized in the following

TABLE 2

Sample	Relative Activity
Solution prior to mixing with hexadecane	1.00
Solution following mixing with hexadecane	0.56
Activity adsorbed to interface following mixing procedure	
Aqueous sample following solidification and melting of hexadecane layer	0.31

Following is a summary of the results illustrated by the foregoing examples:

When a commercial preparation of a mixture of seven marker proteins was vortexed in the presence of hexadecane in accordance with Example 1 above, one of the bands, corresponding to glyceraldehyde-3-phosphate dehydrogenase (G3PD), was almost completely removed from the aqueous phase, as observed by SDS-polyacrylamide electrophoresis of the mixture (FIG. 1, lane a). When the bottom aqueous phase was removed and replaced, and the hexadecane layer subsequently frozen and thawed, the enzyme was clearly eluted (FIG. 1, lane b).

FIG. 2 (lanes a–d) illustrate the absorption and desorption of lysozyme from clarified human saliva assayed on lawns of *Micrococcus lysodeikticus* as described above: (a) zone of inhibition of clarified human saliva; (b) loss of activity following mixing with hexadecane; (c) no significant activity observed in PBS wash of hexadecane layer; (d) reappearance of activity in aqueous phase following solidification and melting of hexadecane layer.

The mixing of whole, clarified saliva in the presence of hexadecane in accordance with Example 2 above brought about a 70% loss in lysozyme activity, as compared with that prior to mixing (Table 1 and FIG. 2). Forty-two percent of the activity lost in the mixing procedure (i.e., 30% of the original activity of the sample) could be recovered from the oil: water interface following phase transition of the hydrocarbon.

As shown in Example 3 and Table 2, lysozyme may be similarly extracted and eluted from PBS. In Table 2, lysozyme activity was measured by applying 25 1 aliquots to paper pads, and measuring their zones of inhibition on lawns of *Micrococcus lysodeikticus* as described above. The Relative Activity was calculated from calibration curve using various salivary dilutions as described above. The loss in activity following mixing was presumably due to adsorption. Seventy percent of the activity removed from the PBS upon vortexing could be recovered from the distilled water following the freezing and thawing procedure. Elution into PBS, however, was less effective.

While the hexadecane is used as the liquid oil, it will be appreciated that other oils could be used, e.g., coconut oil, octadecane, preferably having melting points of −20° C. to 50° C. Coconut oil has a melting point of 21°–25° C., and when used, the extraction should preferably be at about 30° C.

The foregoing results indicate that certain proteins from commercial and biological mixtures can be removed to the aqueous: oil interface following a simple mixing procedure. Moreover, the desorbed proteins can be eluted by solidifying and melting the oil phase, concomitant with droplet coalescence. The finding that up to 70% of the lysozyme activity adsorbed to the aqueous:hexadecane interface could be recovered using this procedure indicates its potential for enzyme separation and purification, such as insulin, α-interferon, γ-interferon, antibody growth hormones.

The above method has several advantages which suggest its potential use in commercial purification techniques: (i) the commercial availability and low cost of hexadecane and other suitable liquid oils; (ii) the large potential interface available for adsorption on the oil droplets as compared with solid supports, and (iii) eluent addition is not required. It is clear that for specific separation purposes, various aspects of the procedure may be optimized, e.g., mixing energy and duration, composition of the aqueous and hydrocarbon phases.

While the invention has been described with respect to several preferred embodiments, it will be appreciated that many other variations, modifications and applications of the invention may be made.

What is claimed is:

1. The method for the separation or purification of biopolymers comprising the steps:

adsorbing the biopolymer on the surface of liquid oil droplets;

and separating the adsorbed biopolymer from the oil droplets.

2. The method according to claim 1, wherein the adsorbed biopolymer is separated from the oil droplets by mixing the droplets in an aqueous liquid, removing the aqueous phase and adding a fresh aqueous phase to the droplets, cooling the mixture to solidify and coalesce the oil and to cause it to release the adsorbed biopolymer to the fresh liquid, separating the fresh liquid and the biopolymer from the coalesced oil, and separating the biopolymer from the fresh liquid.

3. The method according to claim 1, wherein said oil droplets are of a liquid hydrocarbon having a melting point between −20° C. to 50° C.

4. The method according to claim 3, wherein said oil droplets are of hexadecane.

5. The method according to claim 1, wherein said adsorption is effected by mixing an aqueous solution containing the biopolymer with the liquid oil.

6. The method according to claim 5, wherein the aqueous solution is mixed with the liquid oil at room temperature.

7. The method for the separation or purification of biopolymers comprising the steps: adsorbing the biopolymer on the surface of liquid oil droplets; and separating the adsorbed biopolymer from the oil droplets by mixing the droplets in an aqueous liquid, removing the lower aqueous phase and adding a fresh aqueous phase to the droplets, cooling the mixture to solidify and coalesce the oil and to cause it to release the adsorbed biopolymer to the fresh liquid, separating the fresh liquid and the biopolymer from the coalesced oil, and separating the biopolymer from the fresh liquid.

8. The method according to claim 7, wherein said oil droplets are of a liquid hydrocarbon having a melting point between −20° C. to 50° C.

9. The method according to claim 8, wherein said oil droplets are of hexadecane.

10. The method according to claim 8, wherein said adsorption is effected by mixing an aqueous solution containing the biopolymer with the liquid oil.

11. The method according to claim 10, wherein the aqueous solution is mixed with the liquid oil at room temperature.

12. The method for the separation or purification of biopolymers comprising the steps: adsorbing the biopolymer on the surface of liquid oil droplets by mixing an aqueous solution containing the biopolymer with the liquid oil; and separating the adsorbed biopolymer from the oil droplets.

13. The method according to claim 12, wherein the adsorbed biopolymer is separated from the oil droplets by mixing the droplets in an aqueous liquid, removing the lower aqueous phase and adding a fresh aqueous phase to the droplets, cooling the mixture to solidify and coalesce the oil and to cause it to release the adsorbed biopolymer to the fresh liquid, separating the fresh liquid and the biopolymer from the coalesced oil, and separating the biopolymer from the fresh liquid.

14. The method according to claim 12, wherein said oil droplets are of a liquid hydrocarbon having a melting point between −20° C. to 50° C.

15. The method according to claim 14, wherein said oil droplets are of hexadecane.

16. The method according to claim 12, wherein the aqueous solution is mixed with the liquid oil at room temperature.

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Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Harry F. Manbeck, Jr.

Commissioner of Patents and Trademarks

Linda P. Elliott

Attest

NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number, and timing of the maintenance fees required may be changed by law or regulation.

United States Patent [19]

Rosenberg et al.

[11] **Patent Number: 4,976,951**

[45] **Date of Patent: Dec. 11, 1990**

[54] **DENTAL CARIES DIAGNOSTIC AND LOCALIZATION TECHNIQUE**

[76] Inventors: **Melvyn Rosenberg**, 12 Yeda Am, Ramat Gan 52526; **Ilana Eli**, 26 Shazar, Ramat Gan 52247; **Ervin Weiss**, 9 Lipsky, Tel-Aviv 62195, all of Israel

[21] Appl. No.: **770,201**

[22] Filed: **Aug. 28, 1985**

[51] **Int. Cl.**[5] **A61K 49/00**; C12Q 1/00; C12Q 1/24; C12Q 1/04

[52] **U.S. Cl.** **424/7.1**; 424/2; 435/4; 435/7; 435/30; 435/34; 435/36

[58] **Field of Search** 424/7.1, 9; 435/4, 7, 435/36, 30, 34; 514/781; 433/215, 168.1, 213; 378/62; 264/16–20; 106/38.2

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Primary Examiner—Douglas W. Robinson

Attorney, Agent, or Firm—Benjamin J. Barish

[57] **ABSTRACT**

A method for detecting and localizing bacterial growth on a surface comprising the steps of:

(a) applying a solid substrate to the surface;

(b) removing said substrate from said surface and incubating said substrate for purposes of facilitating ultimate visualization of selective bacterial growth; and

(c) observing the locus of bacterial growth on the substrate.

The method is particularly effective in detecting and localizing cariogenic activity in the mouth.

19 Claims, No Drawings

DENTAL CARIES DIAGNOSTIC AND LOCALIZATION TECHNIQUE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a method and apparatus for the early diagnosis and localization of dental caries.

2. Description of Relevant Materials and Background

Until recently, there was little reason to be concerned with attempting to localize the development of dental caries, other than from a theoretical viewpoint. Practically speaking, no techniques were available to arrest the development of dental caries and early information as to their existance was of little practical use.

Recently however, developments in dental science such as the use of polymeric sealants have made it possible to arrest the growth and development of dental caries such that it is now of considerable practical interest to be able to determine and localize the early presence of dental caries so that their further development may be arrested.

Traditional examination techniques such as x-rays and examination with a dental explorer are capable of detecting the presence of dental caries only at relatively late stages of development when the development can no longer be arrested by newly developed techniques. Furthermore, the use of such techniques on a regular basis is limited because of the health dangers associated with radiation, as well as the discomfort, and possible damage to the patient since it has been shown that probing with an explorer damages the dentine in incipient caries. Furthermore, performance of such techniques requires trained personnel and does not allow for detection by the patient himself, for example.

It would, therefore, be very desirable to provide a technique which allows for the early detection and localization of dental caries by a procedure which is simple to perform, even by untrained personnel, and which is not disturbing to the patient, as has traditionally been the case.

Although techniques have now been developed which yield a general measure of cariogenic microorganism activity in the mouth, none of the available techniques actually localizes the site of the cariogenic activity.

STARK, U.S. Pat. No. 3,279,068, discloses a method of detecting carious tissue and dental calculus. The patent discloses that 9-aminoacridine and its hydrochloride are unique agents for disclosing the presence of dental caries and calculus. The patent does not discuss the detection of *Streptococcus mutans* (*S. mutans*) or other cariogenic microorganisms.

GREEN, U.S. Pat. No. 3,332,743, discloses a diagnostic test for dental caries activity. The test gives a color response at room temperature, based on the sum total of metabolic activity of the saliva (column 1, line 60). In accordance with that test, a buffered substrate solution containing a suitable indicator dye measures the oxidation rate of a saliva specimen by measuring the time period in which the indicator dye undergoes a distinct color change, which is taken as a measure of dental caries activity (column 1, line 70). The test does not provide spatial localization of microorganisms associated with the cariogenic activity.

HOERMAN et al., U.S. Pat. No. 3,746,625, discuss a 48-hour test for *S. mutans* in plaque. The method involves removing a series of specimens from the patient's mouth, generally by insertion of dental floss between the teeth to remove material in the mouth adjacent to the tooth (column 1, line 60).

The floss with the specimen on it is dropped into a test solution. After a 48-hour period, a yellow color indicates the presence of *S. mutans* (column 1, line 65 and column 2, line 15).

There is no disclosure of localizing cariogenic activity to a specific area of a tooth. Moreover, dental floss, when inserted between two teeth will naturally sample one surface of each tooth at the same time. Additionally, since cariogenic activity is not limited to *S. mutans*, a test limited to this analyte may not be completely accurate.

STEARNS et al., U.S. Pat. No. 3,903,252, describes a dental disclosing composition to be used in the mouth. A staining agent associated with the composition adheres to plaque on the teeth. However, the presence of plaque on the teeth does not necessarily indicate the presence of microorganisms which cause cariogenic activity. Thus, dental disclosing compositions, as exemplified by this patent, do not localize cariogenic activity.

NAKAMURA et al., U.S. Pat. No. 4,359,455, disclose a diagnostic test composition for dental caries activity. The composition contains a saccharide as a carbon source for promoting the acid-producing capacity of bacteria in the mouth as well as a pH indicator which has a color change point at a pH range of 5 to 7. According to the method, a sample of dental plaque is collected and introduced into the composition, and then the change of color of the pH indicator contained in the composition is observed with the naked eye (column 1, line 52). Thus, the composition constitutes an overall measure of bacterial activity in the mouth, and is not intended to localize the activity.

DEYLOFF, U.S. Pat. No. 4,468,456, discloses a medium for differentiating *S. mutans*. The patent contemplates the innoculation of a culture medium with samples taken from the mouth (column 6, line 30). There is no disclosure showing the localization of cariogenic activity to specific areas of the teeth.

SUMMARY OF THE INVENTION

It is, therefore, an object of the invention to provide a technique for determining the extent and location of cariogenic activity in a manner which can be performed by untrained personnel at no risk, or discomfort to the patient.

According to the present invention, there is provided a method for detecting the presence, extent and location of one or more specified microorganisms on a surface, such as on a person's teeth or in another medium, comprising: (a) contacting the surface with a solid substrate containing at least one substance essential for the growth of the specified microorganism; (b) removing the solid substrate from the surface and immersing it in a liquid growth medium lacking said at least one substance essential for the growth of the specified microorganism but otherwise having the necessary growth ingredients for growing the specified microorganism; and (c) incubating the liquid growth medium with the solid substrate immersed therein to promote the growth of the specified microorganism at the interface between the solid substrate and the liquid medium.

The invention also provides, for use in detecting the presence, extent and location of a specified microorganism in an examined medium, the combination compris-

ing: a solid substrate containing at least one substance essential for the growth of the specified microorganism; and a liquid growth medium lacking said at least one substance essential for the growth of the specified microorganism but otherwise having the necessary growth ingredients for growing the specified microorganism, such that by effecting contact between the examined medium and the solid substrate, immersing the solid substrate into the liquid growth medium, and then incubating the liquid growth medium with the solid substrate immersed therein, the growth of the specified microorganism is promoted at the interface between the solid substrate and the liquid growth medium.

The invention is particularly applicable wherein the examined medium containing the one or more specified microorganisms to be detected is the surface of a subject's teeth, or wherein the solid substrate would preferably be an impressionable material, such as chewing gum, applied to the subject's teeth.

An important feature of the method of the present invention is that it uses a liquid rather than a gel as the growth medium. This enables the method to be conveniently performed by non-professional personnel, even by the patient himself, at no risk or discomfort to the patient.

According to the preferred embodiments of the invention described below, the liquid growth medium includes an indicator substance which becomes associated with the microorganism during the incubation of the substrate in the liquid growth medium to facilitate identification of the specified microorganism; in addition, the substrate, or the liquid growth medium, may also contain at least one substance which inhibits the growth of microorganisms except the specified microorganism. One possible essential substance absent from the solid substrate and present in the liquid is a carbon source such as sucrose, for example. Other substances for the growth of the microorganism which may be present in the liquid comprise: other sugars (as carbon sources), amino acids (carbon, nitrogen and sulphur sources), sulphate ions, phosphate ions, etc.

The technique is most preferably utilized to localize cariogenic activity on a tooth surface. In this embodiment the substrate may be selected from the group consisting of: chewing gum, plant or vegetable gum, alginate, a paper card, a plaster mold, wax, silicone, cellophane, etc.

As noted above, the substrate or liquid growth medium may further, or alternatively, comprise an antibacterial agent to prevent growth of bacteria other than the bacteria of interest. For example, 60% sucrose is inhibitory to noncariogenic microorganisms as well as being a main carbon source.

When utilized in the mouth the technique most preferably involves:

(a) contacting the mouth interior with a substrate in a manner whereby suspected bacteria within the mouth come into direct contact with the substrate, the substrate comprising at least one carbon source important for the growth of the bacteria on the substrate;

(b) removing the substrate from the mouth and incubating the substrate within a liquid medium comprising all additional substances within a liquid medium comprising all additional substances for the growth of the suspected bacteria on the substrate for a sufficient period of time to allow for the growth of the bacteria on the substrate in a manner whereby the locus of bacteria on the substrate is not substantially disturbed upon exposure to the liquid medium; and

(c) observing the growth of the bacteria on the substrate to identify the presence and locus of cariogenic activity in the mouth.

Under certain circumstances it may be necessary to rinse the substrate prior to incubating it.

The liquid medium includes a disclosing agent such as a dye, in one embodiment, such as gentian violet, trypan blue, potassium tellurite, or mixtures thereof, for example, to facilitate visualization of the bacteriological activity.

According to another embodiment, a chromogenic immunoassay system may be utilized; that is, the disclosing agent may be an immune serum containing antibodies to be bound to the bacterium during incubation of the substrate. The antibodies may be labelled to facilitate observation, or may in turn be unlabelled for subsequent coupling with an enzyme-anti-immunoglobulin conjugate.

DESCRIPTION OF PREFERRED EMBODIMENTS

The technique according to the invention provides a map of cariogenic activity on teeth surfaces by enabling growth of cariogenic microorganisms on a solid replica substrate following incubation in a selective growth medium. The substrate is developed to indicate the extent and locus of cariogenic activity.

According to the invention an examination is performed by contacting the surface to be examined with the substrate. When examining the interior of the mouth, the individual teeth, either separately or as a group, are brought into contact with the substrate to transfer cariogenic bacteria from the teeth to the substrate. Known cariogenic bacteria include *Streptococcus mutans, mitis, sanguis, Actinomyces Israeli, viscosus,* and certain lactobacilli. Any technique for transferring the bacteria may obviously be used.

By way of example, a patient may be made to bite on a chewing gum substrate which molds to the configuration of the teeth. The chewing gum itself is also essentially free of antibacterial agents so as to allow the growth of the cariogenic bacteria thereon. In addition to chewing gum, other substrates are quite obviously possible such as, for example, plant or vegetable gum, alginate, a paper card, a plaster mold, wax, silicone, cellophana, etc. The solid replica substrate surface contains at least one component which is essential for subsequent bacterial growth, such as a main carbon source. The carbon source may be, for example, sucrose, galactose, sorbitol, glucose or other sugars, amino acids, sulphates, or alternatively phosphates and ammonium salts. Absence of this at least one essential component limits bacterial growth anywhere other than in contact with the substrate surface, after removal of the substrate.

After contact between the substrate and the teeth has taken place, the replica substrate is separated from the teeth and washed to remove non-bound cells which might interfere with the localization technique. The replica is then placed into a liquid selective growth medium which contains all of the components necessary for growth of cariogenic bacteria, except one essential component which is provided within the solid replica. Thus, if the replica contains the carbon source, it is

absent from the liquid growth medium. Since the other necessary components for growth are present in the liquid medium, growth occurs primarily at the solid-liquid interface.

Other ingredients necessary for proper growth of the cariogenic bacteria are peptides, amino acids, plant and animal extracts, and phosphate. These substances are present in the liquid growth medium.

In addition to containing growth agents, the liquid growth medium or solid substrate may additionally contain components which inhibit the growth of non-cariogenic bacteria. Such materials include, by way of example, potassium tellurite, trypan blue, gentian violet, etc. Likewise the pH of the liquid may also be adjusted to a sufficiently low level as an alternative or cumulative means of inhibiting growth of non-cariogenic bacteria.

An indicator substance is further included, preferably in the liquid growth medium, which is absorbed by the bacteria during their growth. By way of example, such an indicator my include gentian violet. This indicator allows for visualization of the cariogenic activity at its locus. Gentian violet thus serves not only as an indicator, but also as an inhibitor of non-cariogenic bacteria.

Incubation of the solid replica substrate within the liquid medium occurs at temperatures of approximately 35°–37° C. for periods of approximately 6–24 hours sufficient to allow for significant bacterial growth to permit ultimate visualization.

Following incubation of the solid replica within the liquid medium bacterial growth can be easily perceived by absorption of the indicator at certain locations on the replica substrate. This in turn can be correlated with the cariogenic activity of the tooth surface.

Another indication technique which is possible, includes the use of standard immunological reagents which make visualization possible. Such techniques may, for example, include incubating the substrate in an immune serum which contains antibodies which specifically bind with cariogenic bacteria during incubation of the solid replica substrate. If the antibodies are labelled, such as with a chromogenic enzyme, for example, visualization is then possible by subsequently contacting the enzyme with a corresponding substrate. Alternatively, the antibodies may be unlabelled, with the visualization technique including contacting the substrate with a solution of labelled anti-immunoglobulin antibodies which bind to the unlabelled antibodies on the substrate.

EXAMPLE

The process will now be exemplified by way of the following non-limiting example:

The patient is asked to bite down on a solid replica substrate such that a visual imprint of the tooth surface on the chewing gum is made. The gum has the following composition: 20% Paloya gum base (L. A. Dreyfus Co.), 1% glycerol, 60% sucrose, and 19% glucose. The chewing gum may be exposed on only one of its surfaces to the teeth, or may be exposed on both surfaces to the upper and lower teeth. Also, the buceal and lingual sides of the teeth may be exposed.

The chewing gum is then carefully removed from the mouth of the patient, washed briefly in tap water, and exposed to a liquid medium having the following composition: 10 g/l Bactotryptose, 10 g/l proteuse peptone, 75 mg./l trypan blue, 0.8 mg/l gentian violet and 10 ug/l potassium tellurite. The chewing gum is permitted to incubate within the liquid medium for approximately 6–12 hours at a temperature of approximately 37 degrees Centigrade. After incubation, the liquid medium is drained, and the substrate is visually examined for evidence of cariogenically-related microorganisms, which organisms have absorbed the visually-observable dye.

The patient, technician or dentist is then able to identify the locus of the cariogenic activity by correlating the replica with the teeth of the patient.

Thus, although previous techniques have allowed for procedures which merely test for the presence of cariogenic activity, such techniques have not allowed for the localization of the activity and have thus given little more than evidence that a cavity is about to occur. This information would then require the more careful routine examinations which have been traditionally performed and which suffer from the drawbacks mentioned above. Using the technique of the invention the site of the activity is localized and the bacteria may be treated at an early stage using techniques which are now becoming available. Also, the technique involves inexpensive materials which are harmless, unlike x-rays, and does not require peripheral equipment, other than an incubator.

Although the invention has been described with reference to particular means, materials and embodiments, it is to be understood that the invention is not limited to the particulars described and extends to all equivalents. For example, although the invention has been described with reference to chewing gum as the replica substrate, it is clear that other substrates such as cardboard sheets, plaster molds, and the like may be used without going beyond the scope of the invention. Likewise, although the invention has been described with respect to a particular nutrient source in the substrate, it is clear that other nutrient growth sources may be present in either the substrate or the liquid medium to achieve the desired objectives. Finally, although described with reference specifically to tooth examination, it is clear that the localization concept of the invention is not limited to teeth and may be used wherever one desires to determine and localize the existence of suspected bacteria on a surface.

What is claimed is:

1. A method for detecting the presence, extent and location of a specified microorganism on a surface, comprising:

(a) contacting the surface with a solid substrate containing at least one substance essential for the growth of the specified microorganism;

(b) removing said solid substrate from the surface and immersing it in a liquid growth medium lacking said at least one substance essential for the growth of the specified microorganism but otherwise having the necessary growth ingredients for growing the specified microorganism; and

(c) incubating said liquid growth medium with the solid substrate immersed therein to promote the growth of the specified microorganism at the interface between the solid substrate and the liquid medium.

2. The method according to claim 1, wherein: said surface is a subject's teeth, the solid substrate is an impressionable material applied to the subject's teeth, and said specified microorganism is caries-associated bacteria.

3. The method according to claim 2, wherein said impressionable material is chewing gum.

4. The method according to claim 1, including the further step of rinsing the solid substrate with water before immersing it in the liquid growth medium in order to remove non-adherent substances and microorganisms.

5. The method according to claim 1, wherein said at least one substance essential for the growth of the specified microorganism contained in the solid substrate, but lacking in said liquid growth medium, is a carbon source.

6. The method according to claim 5, wherein said carbon source is sucrose.

7. The method according to claim 1, wherein said liquid growth medium includes at least one indicator substance which becomes associated with the microorganism during the incubation of the substrate in the liquid growth medium, to facilitate identification of the specified microorganism.

8. The method according to claim 7, wherein said at least one indicator substance in the liquid growth medium is a chromogenic selective agent absorbed by the microorganism during the incubation of the substrate in the liquid growth medium, to facilitate visual observation.

9. The method according to claim 7, wherein said at least one indicator substance is an immune serum containing fluorescent or enzyme-containing antibodies associated with the specified microorganism during incubation of the substrate in the liquid growth medium.

10. The method according to claim 1, wherein said solid substrate further includes a substance inhibiting the growth of microorganisms except the specified microorganism.

11. The method according to claim 1, wherein said liquid growth medium includes a substance inhibiting the growth of microorganisms except the specified microorganism.

12. A method for detecting the presence of one or more specified microorganisms in an examined medium comprising:

(a) effecting contact between said examined medium and a solid substrate containing at least one substance essential for the growth of the specified microorganism;

(b) immersing said solid substrate in a liquid growth medium lacking said at least one substance essential for the growth of the specified microorganism but otherwise having the necessary growth ingredients for growing the specified microorganism; and

(c) incubating said liquid growth medium with the solid substrate immersed therein to promote the growth of the specified microorganism at the interface between the solid substrate and the liquid medium.

13. The method according to claim 12, wherein: said solid substrate is an impressionable material, and said specified microorganism is caries-associated bacteria.

14. The method according to claim 13, wherein said impressionable material is chewing gum.

15. The method according to claim 12, including the further step of rinsing the solid substrate with water before immersing it in the liquid growth medium in order to remove non-adherent substances and microorganisms.

16. The method according to claim 12, wherein said at least one substance essential for the growth of the specified microorganism contained in the solid substrate, but lacking in said liquid growth medium, is a carbon source.

17. The method according to claim 16, wherein said carbon source is sucrose.

18. The method according to claim 12, wherein said solid substrate further includes a substance inhibiting the growth of microorganisms except the specified microorganism.

19. The method according to claim 12, wherein said liquid growth medium includes a substance inhibiting the growth of microorganisms except the specified microorganism.

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Renewal
June 1997

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Bruce Lehman

Commissioner of Patents and Trademarks

Sandra J. Morton

Attest

NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number, and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

US005270174A

United States Patent [19]

Rosenberg

[11] Patent Number: 5,270,174

[45] Date of Patent: Dec. 14, 1993

[54] METHOD AND KIT FOR INDICATING THE LEVEL OF ORAL MICROBIAL ACTIVITY

[75] Inventor: Melvyn Rosenberg, Ramat-Gan, Israel

[73] Assignee: Assif Science and Technology Projects Development Ltd., Ramat-Gan, Israel

[21] Appl. No.: 329,876

[22] Filed: Mar. 28, 1989

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 174,669, Mar. 29, 1988, abandoned.

[30] Foreign Application Priority Data

Apr. 3, 1987 [IL] Israel 82095

[51] Int. Cl.[5] C12Q 1/22; C12Q 1/04; C12Q 1/02

[52] U.S. Cl. 435/34; 435/26; 435/29; 435/30; 435/31; 435/32; 435/810; 436/1

[58] Field of Search 435/26, 29, 32, 34, 435/810, 31, 30, 296, 287; 436/1, 64, 809; 424/71, 49; 422/102; 206/569, 520, 305; 248/152; 383/36

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Primary Examiner—Toni R. Scheiner

Attorney, Agent, or Firm—Benjamin J. Barish

[57] ABSTRACT

The invention provides a method for indicating the level of oral microbial activity comprising providing a sterile liquid for introduction into the oral cavity for swishing therein, providing a transparent vessel having therein an indicator which undergoes a characteristic color change in the presence of oxygen-consuming microbes and placing the swished liquid together with nutrients to enhance microbial growth in the vessel, whereby the indicator undergoes a color change as a function of time, which color change is an indication of the level of microbial activity in the swished expectorated liquid. The invention also provides a kit for use in carrying out the method comprising a sterile liquid, a transparent vessel for use in receiving the expectorate, and an indicator to be provided in the vessel to produce a visual indication of the level of microbial activity in the expectorated liquid.

7 Claims, 1 Drawing Sheet

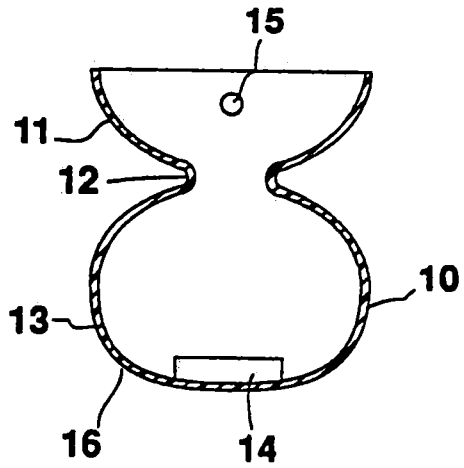

FIG . 1

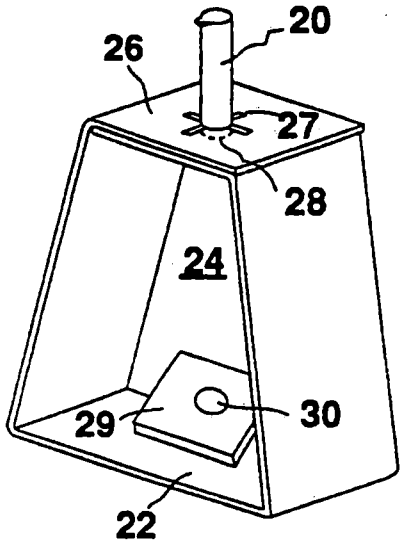

FIG . 2

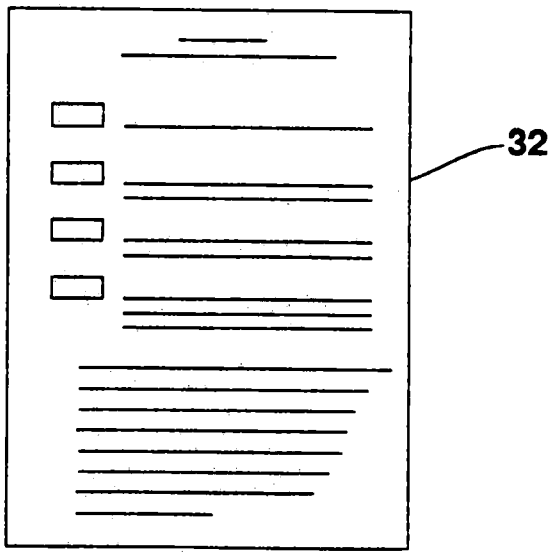

FIG . 3

METHOD AND KIT FOR INDICATING THE LEVEL OF ORAL MICROBIAL ACTIVITY

The present specification is a continuation-in-part of U.S. Ser. No. 07/174,669 filed Mar. 29, 1988, now abandoned.

The present invention relates to a method and kit to ascertain and measure the level of the microbial activity and concomitant clinical parameters in the mouth, by either dental practitioners, or auxiliary staff or by patients themselves.

Many oral ailments (bad breath, caries and periodontal diseases) can be related to microbial activity ill the mouth. Bad breath (halitosis) is usually related to oral accumulation of microorganisms, e.g., subgingival dental plaque, between the teeth, under a prosthesis, on the tongue, etc.

Thus as reported in the literature, increased levels of dental plaque results in dental caries and/or periodontal disease. Whereas dental caries is commonly associated with elevated levels of *Streptococcus mutans* (Loescne, W. J. 1986. Role of Streptococcus mutans in human dental decay. Microbiol. Rev. 50:353-380), periodontal disease is accompanied by elevated levels of more than 57 bacterial species (Moore, W. E. DC., L. V. Holdeman, E. P. Cato, R. M. Smibert, J. A. Burmaister, K. G. Palcanis and R. R. Ranney. 1985. Comparative bacteriology of juvenile periodontitis. Infect. Immun. 48:507-519).

Similarly, halitosis (bad breath) is related to the metabolism of a variety of microorganisms which produce hydrogen sulphide, mercaptans, and other cytotoxic, putrid volatile gases, generally under anaerobic conditions (Tonzetich, J. 1977. Production and origin of oral malador: a review of mechanisms and methods of analysis. J. Periodontol. 48:13-20).

There is little, if any, doubt today that chronic inflammatory periodontal disease results from bacterial plaque accumulation as can be learned, e.g., from Slots J. 1979. Sublingual microflora and periodontal disease. J. Clin. Periodontol. 8:351 and Hiederman van Palenstein, W. H. 1981. Microbial aetiology of periodontal disease. J. Clin. Periodontol. 8:261.

Since as reported, e.g. by Schluger, S., A. Yuodelis and R. Page. 1979 Epidemiology of periodontal disease, pp. 23-87 In Periodontal Disease (1st ed.), Lea & Febiger, Philadelphia, Pa., periodontal disease in its different forms affects most of the world's population, much attention has been given to diagnostic means: bleeding indices, plaque and periodontal indices, clinical probing, etc. Most available techniques require sophisticated skills, and costly doctor's "chair" time. Simple methods which may be carried out by office auxilliaries, yet providing reliable and valid data, are therefore essential.

It is of great benefit to the dental practitioner and to the patient to have at their disposal criteria with which to measure the oral levels of bacteria or other microorganisms (e.g., yeast). Some methods enable assessment of specific organisms in the mouth, such as plating of saliva samples on selective growth media, for estimation of lactobacilli, *Streptococcus mutans* or yeast. However, these methods do not provide an overall picture of oral microbial activity, and give results only after several days. One rapid test is to estimate the level of bacterial accumulation on the tooth surfaces (supergingival dental plaque), using a dye such as erythrosine, but this test

does not provide an overall picture of the microbial activity in the mouth.

An object of the present invention is to provide a simple and convenient method for the measurement of the overall level of oral hygiene, which can be correlated to oral ailments, e.g., bad breath and periodontal disease.

Another object of the invention is to provide a kit for use in practicing the above method.

According to the present invention, there is provided a method for indicating the level of oral microbial activity comprising:

providing a sterile liquid for introduction into the oral cavity for swishing therein, providing a transparent vessel having therein an indicator which undergoes a characteristic color change in the presence of oxygen-consuming microbes and placing said swished liquid together with nutrients to enhance microbial growth in said vessel, whereby said indicator undergoes a color change as a function of time, which color change is an indication of the level of microbial activity in said swished expectorated liquid.

In preferred embodiments of the present invention said sterile liquid is one which contains nutrients to enhance microbial growth and also has good cleansing properties and is preferably selected from sterilized milk, a milk-based liquid or a conventional milk substitute.

Alternatively, said sterile liquid is water and said vessel is provided with an indicator which undergoes a characteristic color change in the presence of oxygen-consuming microbes and with a nutrient containing substance to enhance microbial growth.

According to a further feature of the invention, there is provided a kit comprising a sterile liquid, a transparent vessel for use in receiving the expectorate, and an indicator to be provided in the vessel and to produce a visual indication of the level of microbial activity in the expectorated liquid; the kit preferably also includes a color chart indicating the various levels of microbial activity present when a predetermined color change is produced over a predetermined time interval in all the material in the vessel, or at calibrated levels in the vessel. The color chart can be used to indicate the level of microbial activity by measuring the color change after a predetermined time interval, or by measuring the time interval for a predetermined color change to take place.

According to another feature of the invention there is provided a preferred kit for use in indicating the level of oral microbial activity in accordance with the method herein defined, comprising a sterile liquid having cleansing properties and containing nutrients to enhance microbial growth, a transparent vessel for use in receiving the expectorate, an indicator to be provided in the vessel to produce a visual indication of the level of microbial activity in the expectorated liquid and a stand for receiving and holding said vessel, said stand comprising a supported elevated platform provided with an aperture sized to receive the bottom of said vessel, and a mirrored surface provided directly below said aperture through which mirrored surface the color change in the bottom of said vessel protruding downwardly through said aperture can be indirectly viewed.

Further features and advantages of the invention will be apparent from the description below.

The invention is herein described by way of example only, with reference to the examples set forth below,

and also with reference to the accompanying drawings, wherein:

FIG. 1 pictorially illustrates a vessel which may be included in a kit to be used for practicing the method of the present invention.

FIG. 2 pictorially illustrates components of an especially preferred kit to be used for practicing the method of the present invention, and

FIG. 3 pictorially illustrates a color chart that may also be included in the kit.

As described above, the microbial sample is obtained from the oral cavity by swishing with a liquid. The liquid should have several properties, including the following: (i) it should be sterile prior to its introduction into the oral cavity; (ii) it should have good cleansing properties; (iii) it should not harm the microbial population; and (iv) it should provide nutrients and physiological conditions conducive to microbial activities. Among the liquids tested, sterilized milk has proven a suitable liquid for this purpose. There may also be used other milk-based liquids or milk substitutes.

The liquid may also include a selective agent to indicate certain populations of microbial activity. For example, the liquid could include chloramphenicol, to indicate yeast activity.

A given volume of the sterile liquid (e.g. 10 ml) is introduced into the oral cavity, and the patient is asked to swish vigorously for e.g., 30 seconds. The liquid is then expectorated into a vessel.

FIG. 1 pictorially illustrates one form of vessel that may be used. The vessel **10** comprises a large-diameter upper funnel section so that expectoration is conveniently accomplished. If the microbial activity is to be assessed by oxygen consumption, the funnel section **11** leads, via a small diameter bore **12** through which the liquid can percolate, into a lower compartment **13**. The lower compartment contains, either in liquid or solid form, an indicator **14** of microbial activity. The lower compartment may also contain additional nutrients, such as Brain Heart Infusion Broth or glucose. In the examples given below, the indicator consists of methylene blue, which turns from blue to colorless upon reduction, e.g. exhaustion of oxygen. Other possible indications such as H_2S (hydrogen sulphide) detection, or pH changes, or combinations thereof, could also be used for measuring the level of microbial activity.

As opposed to the conventional cuspidors designed to conceal expectorates, the vessel **10** described here should be transparent, in order to enable visualization of the color changes. It is preferably made of an insulating material, e.g., a plastic such as polystyrene, so that the temperature of ca. 35° C. degrees attained during the swishing procedure is conserved. Ideally, the upper section **11** should be funnel-shaped since the expectorated microorganisms are often aggregated, or adhering to epithelial cells or debris and these should be allowed to fall freely to the bottom **13** of the vessel in order to expedite the results. Vessel **10** may also have a hole **15** in its top section **11** permitting the vessel to be hung on the wall. Its bottom **16** should be flat to permit it to stably rest on a flat horizontal surface, or the vessel could be provided with legs (not shown) for this purpose or placed in a stand.

Alternatively, and preferably as shown in FIG. 2 the vessel is in the form of a test tube **20** and the kit contains a stand **22** supporting by means of an upright member **24**, which can be a rod or wall of a container, an elevated platform **26**. The platform is provided with an aperture **27** sized to receive the bottom **28** of said test tube **20** which protrudes downwardly therethrough. A tilted mirrored surface **29** is provided directly below said aperture **27** in which the reflection **30** of said downwardly protruding bottom portion **28** can be seen so that the color change in the bottom of the test tube can be readily indirectly viewed through said mirror **29**.

A timer may be used to indicate the time required for testing the color change of the liquid. In addition, to facilitate the measurement of microbial activity in the liquid, and hence determine the level of microbial activity indicated by the color change of the liquid, a color chart, shown as **32** in FIG. **3**, is provided to show the different levels of microbial activity. The user may compare, at the end of the incubation period, the color of the liquid at the bottom of the vessel, with the color chart, and thereby read the microbial activity level.

The sterilization process of the liquid can be done by irradiation, ultra-high temperatures, autoclave and such process known per se for this purpose.

EXAMPLE 1

A patient was asked to swish in his mouth, for 30 seconds, a 10 milliliter sample of ultra-high temperature (UHT) sterilized commercial cow's milk, 3% butterfat (Tnuva, Rehovot). The expectorate was collected in a transparent plastic vessel. The expectorate was then serially diluted with sterile milk. To 3 ml of the dilutions, 0.1 ml of a 0.1% aqueous solution of methylene blue were added, and following mixing, allowed to stand at 37° C. The time required for the milk at the very bottom of the test tube to turn from blue (aerobic) to white (anaerobic) was recorded. The results are recorded in the following table:

TABLE 1

Dilution	White after
Undiluted expectorate	10 minutes
1:4	70 minutes
1:16	240 minutes
1:32	360 minutes

This data shows that the time required for the bottom of the sample to turn white increases with decreasing numbers of the microorganisms in the sample.

EXAMPLE 2

A volunteer with poor oral hygiene and bad breath swished 10 ml milk in his mouth as in the previous experiment. The milk was expectorated, mixed immediately with 0.4 ml of methylene blue solution (0.1%), and incubated at room temperature in a transparent plastic vessel. The volunteer then brushed his teeth and was asked to repeat the swishing experiment. Whereas the bulk of the prebrushing suspension turned white after 30 minutes incubation, the suspension following brushing turned white after 65 minutes.

In contrast, the suspension of a volunteer with excellent oral hygiene, obtained at the same time and under the same conditions as that of the volunteer with poor oral hygiene, did not turn appreciably white following 100 minutes incubation.

EXAMPLE 3

The following example shows the correlation between bacterial level in the milk in twenty-nine adult volunteers:

The twenty-nine adult volunteers each swished 10 UHT sterilized 3% milk in their mouths for thirty seconds as in the previous experiment. In each instance, the milk was expectorated, and a sample taken for bacterial counts (colony forming units on Brain Heart Infusion Agar, following two days aerobic incubation at 37° C.). 3 ml of the expectorated sample was immediately transferred into a test tube containing 0.12 ml of a 0.1% aqueous solution of methylene blue. Following several seconds mixing, the test tubes were stoppered and allowed to stand. The time required for the milk within a predetermined volume at the bottom of the test tube to turn from blue to white was recorded. The results, summarized below, clearly show that the duration of the color change is inversely related to the microbial count. For example, nine of eleven samples with more than 20,000,000 cfu/ml exhibited a color change within one hour; eleven of twelve samples with less than 10,000,000 cfu/ml exhibited a color change following more than an hour. When the logarithm of the microbial count is plotted against the logarithm of the duration, a high significant correlation (probability <0.001) is obtained, with a correlation coefficient (r) value of -0.849.

TABLE 2

Distribution of Results:		
	Time required for color change	
Number of samples yielding	Less than 60 min.	More than 60 min.
0–10,000,000 cfu/ml	1	11
10,000,000–20,000,000 cfu/ml	4	2
more than 20,000,000 cfu/ml	9	2

As can be seen from the above table the number of samples with rapid color change (less than 60 min) increases as the number of microbes goes up. Conversely, the number of samples turning white after more than one hour increases as the number of microbes goes down.

Statistical analysis of the data reveal that there is a highly significant correlation between the decrease in the time required for the color change (indicative of a high rate of oxygen consumption) and the increase in number of microorganisms in the sample.

EXAMPLE 4

One important aspect of the test is its potential correlation with clinical parameters. A study was performed with 49 volunteers attending a private periodontal clinic. Forty-three of the volunteers were graded according to the presence of moderate or severe periodontal disease (as opposed to no periodontal disease).

Forty-nine patients in a private periodontal practice participated in the study. These patients received periodontal treatment at different stages, i.e. before periodontal examination, prior to or during initial therapy, after initial therapy, and following periodontal surgery. The age and sex of each patient were available. In addition, the last time prior to the milk test at which patients ate or drank was recorded. The periodontal status of each patient was determined according to the *Gingival Index* and *Periodontal Index*, Russell, A. L. 1956, A system of classification and scoring for prevalence surveys of periodontal disease, J. Dent. Res. 35:350, and the plaque level was scored using the *Turesky Plaque Index*, Turesky, S., N. Gilmore and I. Glickman, 1970. Reduced plaque formation by chlormethyl analogue of vitamin C. J. Periodontol. 41:41, on a scale of 0–4.

Each patient swished his mouth for 30 sec with 10 ml of sterile, ultra high temperature sterilized 3% milk (Tnuva, Rehovot) provided with the kit of FIG. 2. The expectorate was collected into a small cup; 3 ml of the expectorate were immediately removed into a disposable test tube (Costar, 10 mm diameter, 12 mm length), containing 0.12 ml of methylene blue (0.1% aqueous solution). The test tube was capped, mixed briefly, placed in an aperture **27** having a diameter of 6 mm of platform **26** of stand **22** and allowed to stand over a tilted mirror **29**. The time required for a color change (blue-to-white) within a 6 mm diameter circle drawn on the bottom of the tube was recorded. The correlation between Plaque Index levels and the logarithm of time required for the color change were calculated by linear regression analysis; similar correlations were established for age of the patient and milk test results. The correlations between periodontal status and milk test results were analyzed using the t-test for sample populations.

The relationship between the time required for color change, and plaque index and the statistical relationships between the time required for color change, and other parameters, is summarized in Table 3. When all 49 patients were considered together, linear regression analysis of the logarithm of time required for color change as function of Plaque Index yielded a correlation coefficient of -0.579 ($p<0.001$). However, when the nineteen patients who ate or drank within 90 minutes prior to the test were excluded, the correlation coefficient increased to -0.641 ($p<0.001$); the correlation coefficient of only those who ate or drank within 90 minutes of the test was -0.481 ($p<0.05$).

TABLE 3

Statistical Data Summary 1. Linear Regression Analysis				
Population	Parameter	number (n)	correlation coefficient (r)	p<
Patients checked	Plaque index	49	−0.579	0.001
Excluding those who ate or drank prior to test	P.I.	30	−0.641	0.001
Only those who ate or drank prior to test		19	−0.481	0.05
Patients	Age	49	−0.337	0.02

TABLE 4

Group	No. of volunteers	Average Time for Color Change (min)
No periodontal disease	30	227
Moderate periodontal disease	8	96
Severe periodontal disease	5	79
Not assessed	6	—

Among 43 patients who were scored for periodontal disease status (five were not scored), 8 were recorded as having moderate gingivitis and 5 as severe gingivitis. Whereas the means time for the color change was 227 min for the control (no periodontal disease) group ($n=30$), the mean times for the moderate ($n=8$) and severe ($n=5$) groups were only 96 and 79 min, respectively. Despite the small numbers of patients with dis-

eased states, both moderate and severe gingivitis groups differed significantly as compared to the control group. The significance level was less for severe vs. control ($p<0.05$) than for moderate vs. control ($p<0.025$), probably due to the smaller number of patients in the severe category.

CONCLUSIONS

1. The test proved to be simple and rapid, requiring no special skills.
2. High correlations with clinical parameters (Plaque Index and periodontal status) were found.
3. The test was liked by the patients who were impressed by the proficiency level of treatment.

Statistical evaluation showed that both moderate and severe periodontal disease groups differed significantly as compared to the control group.

EXAMPLE 5

A volunteer swished his mouth as described in Example 1. The expectorate was diluted serially using sterile milk (prewarmed to 37 degrees Celsius). To test tubes containing 0.12 ml of a 0.1% aqueous solution of methylene blue were added 3 ml of expectorate (or dilution thereof). The test tubes, containing the original expectorate and various dilutions, were allowed to stand at room temperature. The times required for color change (blue-to-white) within 6 mm diameter at the bottom of each test tube were recorded. Microbiological counts (colony forming units) were determined. The correlation between the time required for the color change and the microbial levels in the dilutions are shown below:

TABLE 5

Millions of colony forming units/ml	Time for color change (min)
30	6
21	16
15	22
6	60
3	120
0.21	290

If one plots the logarithm of the number of microorganisms in the sample as a function of the logarithm of the time required for the color change, a straight line is obtained. Statistical evaluation yields an extremely significant correlation between the microbial level in the sample and the time required for the color change at the bottom of the test tube.

EXAMPLE 6

One commonly used method for improving oral hygiene at home is use of an active mouthwash preparation. Two experiments were performed in order to see whether use of such a preparation resulted in concomitant changes in the results of the test. Volunteers were asked to swish for two consecutive 15 second periods with either a potent commercial mouthwash (Listermint, Warner and Lambert, Eastleigh, Hampshire, U.K.) as opposed to a placebo mouthwash (i.e. no active ingredients) (Agis Ltd., 26 Marmorek Street, Tel Aviv, Israel). Prior to the swishing and 30 minutes following swishing, volunteers performed the test as described in the previous example. The results are presented below:

TABLE 6

Volunteer Number	Time required for color change (minutes) MOUTHWASH before	MOUTHWASH after	PLACEBO before	PLACEBO after
1	39	1010	—	—
2	76	530	—	—
3	44	312	—	—
4	18	449	—	—
5	76	338	—	—
6	—	—	60	118
7	—	—	99	76
8	—	—	44	89
9	—	—	96	82
10	—	—	13	59
11	—	—	32	54

As can be seen from the results, in each case swishing with the active mouthwash brought about a large increase in the time required for the color change, as opposed to before using the mouthwash. On the average, the time required increased by a factor of 13.9 in the mouthwash group. On the other hand, the group usng the placebo showed a small increase, by a factor of only 2.03 on the average. Statistical analysis shows that these are highly significant differences, and thus shows that the test correlates with a clinical procedure.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

What is claimed is:

1. A method for indicating the level of oral microbial activity, comprising the steps of:
   (a) providing a sterile liquid containing nutrients to enhance microbial growth, for introduction into the oral cavity for rinsing therein, said sterile liquid being selected from the group consisting of milk, a milk-based liquid and a milk substitute;
   (b) providing a transparent vessel having therein an indicator which undergoes a characteristic color change upon exhaustion of oxygen, in the presence of oxygen-consuming microbes;
   (c) having a patient rinse his or her oral cavity with said sterile liquid and then expectorate the resultant rinse liquid; and
   (d) placing said expectorated rinse liquid in said vessel,

whereby said indicator undergoes a color change as a function of time, which color change is an indication of the level of microbial activity in said expectorated rinse liquid.

2. The method according to claim 1, wherein the indicator is methylene blue which turns from blue to white upon the consumption of oxygen by the microbes within the expectorated liquid.

3. A kit for use in indicating the level of oral microbial activity, comprising:
   a sterile liquid selected from the group consisting of milk, a milk-based liquid, and a milk-substitute;

a transparent vessel for use in receiving an expectorate which consists essentially of the resultant liquid obtained by rinsing a patient's oral cavity with said sterile liquid and expectorating the liquid and entrained oxygen-consuming microbes;

an indicator which undergoes a characteristic color change upon exhaustion of oxygen, in the presence of oxygen-consuming microbes, to be provided in the vessel to produce a visual indication of the level of microbial activity in the expectorated liquid;

and a color chart indicating the various levels of microbial activity present when a predetermined color change is produced over a predetermined time interval.

4. A kit according to claim **3** wherein said vessel is of transparent material and includes an upper funnel section for receiving the expectorate, said funnel section communicating with a lower compartment containing the indicator and in which the test is performed.

5. A kit according to claim **3** wherein said vessel is of polystyrene.

6. A kit according to claim **3**, wherein the vessel includes calibration markings defining a volume at the bottom of said vessel in which the level of microbial activity of the expectorate contained in said defined volume can be determined by measuring the time required for a predetermined color change to appear in said defined volume.

7. A kit for use in indicating the level of oral microbial activity according to claim **3** wherein said vessel is a test tube and further comprising a stand for receiving and holding said vessel, said stand comprising a supported elevated platform provided with an aperture sized to receive the bottom of said vessel, and a mirrored surface provided below said aperture through which mirrored surface the color change in the bottom of said vessel can be indirectly viewed.

* * * * *

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À TOUS CEUX QUI LES PRÉSENTES VERRONT :

Attendue qu'une requête a été présentée au Commissaire aux brevets, demandant la délivrance d'un brevet pour une invention nouvelle et utile, dont le titre et la description apparaissent dans le mémoire descriptif dont copie est annexée aux présentes et en fait partie intégrale, et que ladite requête satisfait aux exigences de la Loi sur les brevets.

À ces causes, le présent brevet confère au demandeur pourqui le titre de propriété dudit brevet est établi d'après les dossiers du Bureau des brevets dont le nom indiqué dans ladite copie du mémoire descriptif ci-annexé, et aux représentants légaux dudit demandeur, pour une période de dix-sept ans, à compter de la date des présentes, le droit, la faculté et le privilège exclusif de fabriquer, de construire, d'exploiter et de vendre à d'autres au Canada l'invention, sauf jugement en l'espèce par tribunal de juridiction compétente.

La concession faite par les présentes étant soumise aux conditions contenues dans la loi précitée, et sous réserve du paiement des taxes réglementaires.

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Whereas a petition has been presented to the Commissioner of Patents praying for the grant of a patent for a new and useful invention, the title and description of which are contained in the specification of which a copy is hereunto attached and made an integral part hereof, and the requirements of the Patent Act having been complied with.

Now therefore the present patent grants to the applicant whose title thereto appears from the records of the Patent Office and as indicated in the said copy of the specification attached hereto, and to the legal representatives of said applicant for a period of seventeen years from the date of these presents the exclusive right, privilege and liberty of making, constructing, using and vending to the others in Canada the invention, subject to adjudication in respect thereof before any court of competent jurisdiction.

Provided that the grant hereby made is subject to the conditions contained in the Act aforesaid, and subject to the payment of maitenance fees.

BREVET CANADIEN 1334173 CANADIAN PATENT

Date à laquelle le brevet a été accordé et délivré	1995/01/31	Date on which the patent was granted and issued

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(19)(CA) **CANADIAN PATENT** (12)

(54) Composition for Desorbing Bacteria

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(30) (IL) Israel 85934 1988/03/30

(57) 14 Claims

NO DRAWING

The present invention relates to novel compositions which desorb bacteria and other microorganisms from solid surfaces and from living tissue.

The main use contemplated for the compositions of the present invention is in oral hygiene. Compositions of the invention can also be used in entirely different applications, where microorganisms are to be removed from surfaces to which they are attached, or where enhanced microbial adhesion to oil droplets is desired.

According to the present invention there is now provided a composition for desorbing bacteria from solid surfaces and from living tissues, which is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime said composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase, comprising a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydrocarbon or a mixture thereof; and

c) about 0.003 to about 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

Amongst edible oils, which can be used to form said water immiscible oily phase there may be mentioned oils such as olive oil, corn oil, coconut oil, soybean oil, safflower oil. There can also be used a wide variety of pharmacologically acceptable hydrocabons such as octane, decane, tetradecane, hexadecane, xylene, white mineral oil, and mixtures thereof etc.

Amongst suitable colors, there may be mentioned colors of triphenylmethane, naphthol, xanthene, monoazo, pyrazol, anthraquinone and cationic colors; examples include Food Blue 2 and its ammonium salt; D&C Yellow No. 7, D&C Yellow No. 10, D&C Yellow Nos. 4, 6, 22, 28, 33 or 40; D&C Green No. 5, D&C Orange No. 11, D&C Red Nos. 19 and 37, Basic Blue Nos. 6, 9, 41, 99, etc.

Preferably said amphipathic cationic moiety is selected from pyridinium core surface-active cationic molecules such as cetylpyridinium chloride, laurylpyridinium chloride, etc.; from chlorhexidines such as chlorhexidine, its diacetate, chlorhexidine digluconate, chlorhexidine dihydrochloride; from monalkyl quaternary ammonium compounds (quats) such as benzalkonium chloride, cetalkonium chloride and bromide, lauralkonium chloride and bromide, soytrimonium chloride, PEG-5 stearyl ammonium lactate; from dialkyl quaternary ammonium compounds (diquats) such as dilauryl dimonium chloride, dicetyl dimonium chloride and bromide, dequalinium chloride, soyamido propyl benzyldimonium chloride, quaterniums such as quaternium 15 and

polyquaterniums, etc; amine fluorides, from cationic polysaccharides, such as chitosan and its derivatives; from cationic polypeptides, such as poly L-lysine, poly D-lysine, lysozyme.

In especially preferred embodiments of the present invention said amphipathic cationic moiety is selected from cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme.

The invention also provides a method for enhancing microbial adhesion at an oil-water interface comprising admixing an aqueous microbial suspension and oil in the presence of an amphipathic cationic moiety.

In U.S. Patent No. 4,525,324, Weiss et al. have described an essentially detergent free mouthwash based on a combination of an oily phase and an aqueous one. This composition must be marketed in a two-compartment squirt bottle, and the user swishes the two phases in his mouth in order to attain the desired effect. The composition is based on the attachment of amphipathic substances to the oil droplets during the swishing action, thus coating the oil droplets. The presence of detergents is reported to be deleterious in said patent. Various deleterious physiological effects have been reported regarding the use of detergents in the oral cavity. It has been reported that the presence of detergents prevents, or at least decreases adhesion of

microorganisms to oil droplets as is taught, e.g. by the statement: "The aqueous bacterial suspension must be free of surfactants, which inhibit the assay" (M. Rosenberg, 1984. Bacterial Adherence to Hydrocarbons: A Useful Technique for Studying Cell Surface Hydrophobicity. FEMS Microbiology Letters 22, 289-295). In a recent review on hydrophobic interactions and adhesion, the authors write: "Our appreciation of the extent to which hydrophobic interactions mediate various bacterial adhesion phenomena could be greatly augmented by including agents that interfere with hydrophobic interactions (e.g., surfactants, chaotrophic agents) in adhesion assays (M. Rosenberg and S. Kjellerberg, 1986, Hydrophobic Interactions: Role in Bacterial Adhesion. Advances in Microbial Ecology 9, 353-393). Moreover, in a previous study which did examine the effect of some cationic amphipathic antibacterial agents on adhesion of relatively non-hydrophobic Escherichia coli strains to xylene, the cells were exposed to the cationic agents, but the cationic agents were subsequently removed by washing the cell pellet prior to addition of the xylene, presumably to avoid inhibition of the adhesion in the presence of the cationic agents (B.M.A. El-Falana, D.T. Rogers, A.D. Russell and J.R. Furr. 1985 Effect of Some Antibacterial Agents on the Hydrophobicity of Wild-Type and Envelope Mutant of Escherichia coli. Current Microbiology 12, 187-190). Similarly, chlorhexidine, an amphipathic, surface-active cationic agent commonly incorporated into mouthwashes has been shown in many studies to inhibit microbial adhesion to surfaces. For example, J.

McCourtie, T.W. MacFarlane and L.P. Samaranayake (Effect of saliva and serum on the adherence of Candida species to chlorhexidine-treated denture acrylic, J. Med. Microbiology, 21:209-213, 1986) showed that treatment of saliva- or serum-coated acrylic with chlorhexidine gluconate reduced adherence by between 19 and 86%.

Moreover, whereas salts such as sodium chloride have been generally considered to enhance microbial adhesion to surfaces, it has now been found that the presence of inorganic cations, such as sodium and magnesium cations, inhibit the stimulation of adhesion brought about by cationic moieties such as cetylpyridinium, chlorhexidine, chitosan, poly L-lysine, poly D-lysine and lysozyme.

The main use of the compositions is in the form of mouthwashes, which effectively remove a large percentage of microorganisms, debris and other odor-causing materials from the surface of teeth and from the oral cavity. Thus especially preferred embodiments of the present invention relate to a mouthwash as defined above comprising about 0.003 to about 0.5 w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

The novel compositions comprise, in combination, an organic water-immiscible phase and an aqueous phase, and a small quantity of an amphipatic cationic moiety, which is adequate to form upon vi orous mixing an emulsion of brief life-time. In addition, the composition may comprise additional cationic agent or agents which enhance adhesion to oil droplets, as shall be illustrated later. Optimally, the composition should be relatively free from interfering cations, such as sodium, potassium and magnesium and therefore preferably contain only up to 0.3% of inorganic salts.

In use, the two phases are mixed by shaking, and an emulsion is formed which is used for swishing in the mouth, and which has a limited life time, of the duration of about 10 seconds to about 30 minutes. A small amount of amine fluoride which tends to stabilize the emulsion for a brief period of time and has other beneficial (e.g. anticaries, antibacterial) effects may also be added.

There may also be incorporated certain additives such as fragrances, colors and the like.

While the invention will now be described in connection with certain preferred embodiments in the following examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives,

modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

The following experimental results illustrate the invention, both in regard to the specific formulations and also as regards the mechanism of action of the emulsions of the present invention as regards removal of dental plaque and adhering bacterial and other microorganisms .

The mouthwash compositions of the invention preferably comprise a small quantity of an amphipatic cationic moiety which is preferrably a surface-active agent which is able to form an emulsion of limited life time. The emulsion ought to be formed upon shaking of the container which contains the two separate phases, and it should preferably remain in emulsion form for at least about 20 seconds. Experiments have shown that compositions of the invention form temporary emulsions which separate after a period of about 10 seconds to 10 minutes.

We have found the unexpected result that small amounts of cationic surface-active agents promote, rather than inhibit, adhesion of bacteria and oral debris, to oil droplets. We have also found the surprising and unexpected results that sodium and magnesium chloride inhibit, rather than enhance, microbial adhesion in the presence of such cationic agents as cetylpyridinium chloride, chlorhexidine digluconate and chitosan.

The following experiments demonstrate the ability of the cationic surfactant, cetylpyridinium chloride, to enhance microbial adhesion to oil droplets in various experiments. In one experiment Acinetobacter calcoaceticus RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30 C in brain heart infusion broth. The cells were harvested by centrifugation and washed twice in 0.2% saline. The cells were then suspended in 0.2% saline to a corrected optical density of about 15 at 400 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% detergent in water (or water for control) and 0.24 ml water. Following brief mixing, 0.2 ml of oil was added (vs. controls with no oil added) and the mixtures vortexed vigorously for 2 min. Following about thirty minutes, the corrected turbidity of the lower aqueous phase was measured at 400 nm. Adhesion is calculated as the percent drop in turbidity following the mixing procedure. The results are summarized in the following Table.

Table 1. Adhesion RAG-1 to various oils in the presence of cetylpyridinium chloride

Additions	Percent adhesion to:		
	n-nexadecane	mineral oil 50	soybean oil
Cetylpyridinium chloride	83	80	47
None	27	5	32
Sodium dodecyl sulphate	0	0	0
Tween 20 *	0	0	0

As can be seen from the above Table, in each case, cetylpyridinium chloride greatly enhanced bacterial adhesion to the oil droplets. This phenomenon was also observed microscopically. Enhanced adhesion was not observed for non-cationic surface-active agents: adhesion to all three test oils in the presence of the anionic sodium lauryl sulphate or the nonionic Tween 20,* added at the same concentration as the cetylpyridinium chloride (w/v), was 0%. Morever, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 98% when 0.08% cetylpyridinium chloride was present; adhesion of the yeast Candida alpicans rose from 20 to 100% in the presence of 0.16% chlorhexidine digluconate. Adhesion of Acinetobacter calcoaceticus MR-481 rose from 0 to 97% in the presence of 0.18% of cetylpyridinium chloride.

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In another experiment, a volunteer swished his mouth with 0.2% saline solution (10 ml) for thirty seconds. To 1 ml of the expectorate were ad ed 0.18 ml of 0.5% cetylpyridinium chloride (water in the case of the control) and 0.44 mm 0.2% saline. Mineral oil 50 (0.2 ml) was then added and the mixtures vortexed for 2 minutes. Following phase separation, the mixtures were examined. Whereas the turbidity of the expectorate was greatly reduced in the presence of cetylpyridinium chloride, presumably due to adsorption of bacteria and debris to the oil droplets, in the absence of this detergent, no increase in turbidity was observed.

The ability of another cationic surface-active agent, chlorhexidine digluconate, to enhance microbial adhesions to oil droplets is shown in the following experiment. Actnetobacter calcoaceticus RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30 C in brain heart infusion broth. The cells were harvested, washed by centrifugation, and suspended in 0.2% saline to a corrected optidal density of ca. 18 at 0 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% chlorhexidine digluconate in water (or water for control) and 0.24 ml water. To each cuvette 0.2 ml of oil was added (vs. control with no oil added) and the mixtures vortexed vigorously for 2 min. After about thirty minutes, the turbidity of the lower aqueous phase was measured at 400 nm and corrected for non-linearity by a standard curve. Adhesion

is calculated as the percent drop in corrected turbidity following the mixing procedure. The results are summarized in the following Table.

Table 2. Adhesion of RAG-1 to various oils in the presence of chlorhexidine digluconate

Additions:	Percent adhesion to n-hexadecane	isohexadecane	Sigma* soybean oil	Commercial soybean oil
Chlorhexidine digluconate	95	94	90	89
None	50	66	54	49

* St. Louis, Mo.

As can be seen from the above Table, in each case chlorhexidine digluconate greatly enhances bacterial adhesion to the oil droplets. Moreover, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 98% when 0.1% chlorhexidine digluconate was present.

Adhesion to oil droplets may also be enahnced by polymeric cationic agents, such as chitosan. For example, the adhesion of Escherichia coli CSH 57 to hexadecane could be increased from 0 to 97% when 0.015% chitosan was present; adhesion of the yeast Candida albicans rose from 20 to 95% in the presence of 0.025% chitosan. Adhesion of Acinetobacter calcoaceticus MR-481 rose from 0 to 99% in the presence of 0.019% of chitosan.

The compositions of the invention are suitable for a variety of applications. As a two-phase mouthwash, containing an aqueous phase and an oil phase, the presence of adhesion-enhancing cationic agent (or combination thereof) could remove and bind high levels of oral microorganisms and debris.

The enhanced hesion of RAG-1 cells to oils in the presence of cationic surfactants similarly teaches the use of cationic surface-active agents to enhance microbial adhesion to oils for commercial purposes, such as immobilization of cells onto oil droplets, or of cells, cell walls, and the like for enhanced adhesion to adjuvant oils in the vaccination field.

In general, salts such as sodium chloride, magnesium chloride and various phosphates, enhance adhesion of microorganisms to surface by countering the net negative charges which generally exist on cell surfaces, and often on substrata surfaces. Weiss et al. include in their U.S. patent 4,525,342, a requirement for salt. However, as stated, it has now been found that the enhancement of microbial adhesion to oils observed in the presence of cetylpyridinium chloride, chlorhexidine gluconate and chitosan is inhibited by sodium chloride and magnesium chloride.

For example, adhesion of C. albicans to hexadecane in the presence of 0.16% cetyl pyridinium chloride fell from 100% to 18% when 0.8% NaCl was present.

In order to obtain a picture as to the efficacy of the compositions of the invention for oral use these were compared with existing mouthwashes as regards their ability to remove a given bacterial film from a solid surface.

There were used a number of commerical products and these were compared with the preparation of the invention defined below as ODEX-35. The following describes and illustrates in detail specifric features of the invention:

Example ODEX-35

Aqueous phase:	
sodium chloride -	0.2% w/w
sodium saccharin -	0.05% w/w
FD&C Blue No. 1 -	0.0001% w/w
cetyl pyridinium chloride -	0.03% w/w
water -	to 100%
Oil phase	
soybean oil -	35.0% w/w
essential oil (mint) -	1.5% w/w

This type of formulation has several advantages. First, the presence of cetylpyridinium chloride results in adsorption of much higher levels of bacterial and oral debris, than when it is absent. Second, the mixture can be presented in a single, regular container, e.g. bottle, obviating the need for a double compartment, double squirt bottle. The user simply shakes for several seconds, the amount of cetylpyridinium chloride is sufficient to provide an emulsion with limited stability which enables the user to pour the desired quantity of mixture and use it directly to swish and gargle. An added advantage of the formulation is that the cetylpyridinium chloride enhances the dye binding capacity of the bacteria and debris so that they are clearly colored blue by the dye, facilitating their observation.

Moreover, the dye and cetylpyridinium chloride may act together in a synergistic fashion as antibacterial agents.

In one experiment, the formulation was mixed, and 10 ml immediately poured into a small cup. Volunteers swished and gargled with the mixture for a thirty second period. Following swishing, the mixture was expectorated into test tubes. It was clearly observed in all cases that the oral bacteria and debris, dyed blue, rose together with the yellow oil droplets, creating a green upper oil:in:water emulsion which was stable for hours. Bacteria, and bacteria-coated epithelial cells were microscopically observed adhering to the oil droplets. The aqueous phase lost its blue color, presumably due to the binding of the dye to the great quantities of bacteria and debris removed onto the oil droplets during the mixing procedure. A drop in the level of bad breath of the volunteers was evident for hours following the swishing procedure. Moreover, volunteers reported a long lasting fresh feeling in their mouths, and did not report any oily taste, despite the high level of oil in the preparation. These observations could not be reproduced if either the oil phase, or the cationic surface-active agent were absent.

It should be emphasized that the compositions of the invention also have a wide range of uses in enhanced desorption of microorganisms for host, industrial and environmental surfaces onto oil droplets. To illustrate this point, we have succeeded in

demonstrating that the above formulation is highly effective in removing adsorbed bacteria from a model surface, i.e. polystyrene, as described in the following experiment.

One ml of a very turbid suspension of RAG-1 cells in 150 mM phosphate buffer was added to a polystyrene cuvette (4 ml capacity). Following 1 minute vortexing, the RAG-1 cells were poured off, leaving a film of adherent cells. The cuvettes were washed briefly with tap water. To each cuvette, 1 ml of mouthwash (when indicated, diluted according to instructions) or water (control) were added and the cuvettes vortexed for 2 minutes. The mouthwash was poured off, 1 ml fresh mouthwash was added, and the cuvettes again vortexed for 2 minutes. The mouthwash was poured of, and the remaining bacterial film stained by adding Gentian Violet to the cuvettes, followed by washing to remove excess stain. The amount of bacterial film was ascertained by measuring the optical absorbance (585 nm) of the dried cuvettes from both sides in a Uvikon spectophotometer. The results are presented in Table 3.

From the following Table it can be seen that among the commercial mouthwashes tested, several (Eludril, P.F. Medicament, 125, rue de la Faisanderie, Paris, France; Tayadent, Taya Ltd., Petach Tiqua, Israel and Act^{R}, Johnson & Johnson, New Brunswick, N.J. 08903) were less able than water to desorb the bacterial film. Menthol Chloraseptic^{R} (Norwich Eaton Pharmaceuticals, Inc., Norwich, N.Y. 13815) and

Veadent^{R} (Glaxo, under licence from Vipont Laboratories Inc., Ft. Collins, U.S.A.)desorbed less than 10% of the adherent bacteria. Cepadont removed 29% of the film. Only ODEX-35 removed the large majority (87%) of the bacterial film. Moreover, ODEX-35 without the oil phase was relatively ineffective (26%) as compared to both phases together (87%).

Table 3

Mouthwash	OD_{585nm} (mean)	Percent removal relative to water control
Control (water	0.300	-
Eludril	0.625	-
Tayadent	0.376	-
Act (green)	0.344	-
Menthol Chloraseptic	0.288	4
Veadent	0.281	6
Cepadont	0.213	29
ODEX-35, aqueous phase only	0.223	26
ODEX-35	0.039	87

In order to further test compositions of the kind described above for use as mouthwashes, several in vivo tests were undertaken. In one of these, various compositions were prepared and swished for two

thirty second periods by a volunteer at bedtime. The following morning, the volunteer swished with ten ml of sterile milk, and 3 ml of the expectorate was mixed in a test tube with 0.12 ml of 0.1% methylene blue. The test tube was allowed to stand at room temperature and the time required for the bottom of the test tube to turn white was observed. It has been previously shown that the time required for a color change from blue to white at the bottom of the test tube correlates highly with microbial counts, thus a table was used to convert the time for color change into relative microbial counts. The results are presented in Table 4, together with the time required for the color change, and the percentage of microorganisms remaining, as compared to a no-mouthwash control. Several of the compositions could not be mixed or were unattractive (samples 3 and 14) and were not tested in the mouth. Others (samples 16 and 23A) were tried several times.

Table 4

Sample Number	1	2	3	4	5	6	12	13	14	15	16	22A	23A	24A	25	26	31	32	34	35	36
Ingredients (%w/w)																					
Soybean oil	35	35	35	35	35	35	35	35	35	35	35	25	15	5	-	-	-	5	33	35	35
Mineral oil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15.0	-	-	-	-
Peppermint oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	0.1	0.1	1.5	0.75	1.5	1.5	1.5
Cetyl pyridinium chloride	0.05	0.025	-	0.025	-	-	-	-	-	-	0.05	0.05	0.05	0.05	-	0.05	0.05	0.05	-	0.05	0.05
Sodium lauryl sulphate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-
Chlorhexidine digluconate	-	-	0.04	0.04	0.2	0.2	0.2	0.1	0.05	0.1	0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	-	-	-
Sodium saccharin	0.05	0.05	0.05	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.05
Xylitol	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aspartame	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-
Sodium chloride	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
Ethanol (absolute)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.0
Tween 20 *	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	0.4	-	-	-	-
FD&C Blue No. 1 (Food Blue 2; CI 42090)	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
Water	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 100%	to 200%
Results:																					
Time for color change	30'	14'	-	10'	>80'	90	420'	>100'	35'	-	>124'	>160'	>120'	>142'	>264'	>226'	129'	<30'	32'	35'	19'
% residual micro-organisms	36.0	61.0	-	77.0	<18.0	17.0	6.0	<15.0	32.0	-	13.0	11.0	<14.0	<12.0	<8.0	<9.0	13.0	>36.0	34.0	17.0	<14.0	36.0	32.0	49.0

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It is clear from Table 4 that a positive interaction exists between cetylpyridinium chloride and chlorhexidine. Thus, whereas sample 1 containing only cetylpyridinium chloride (0.05%) as cationic agent and sample 13 containing only chlorhexidine digluconate (0.05%) as cationic agent left a high percentage of residual microorganisms in the mouth (over 30%), a combination of 0.05% cetylpyridinium chloride and 0.05% chlorhexidine (sample 16) was highly effective, leaving less than 14% residual microorganisms in one instance, and 11% in another. Moreover, the cetylpyridinium chloride has a better emulsifying ability, whereas chlorhexidine digluconate has better antibacterial activity. Thus, the combination of both together with the oil is highly effective in obtaining a composition which can be effectively emulsified by shaking for a brief period, combined with potent antibacterial ability. It should be noted that compositions containing from 5-35% (w/w) of soybean oil, cetylpyridinium chloride, and chlorhexidine were highly effective (samples 15,16, 22A-24A). Another advantage of the two-phase oil:water combination is that moieties which generally require emulsification in regular mouthwashes can be added to the oil phase. For example, in order to introduce peppermint oil into the aqueous phase, it must be solubilized e.g. with Tween.* This causes a great reduction in activity of the formula, by inactivating the cations (e.g. chlorhexidine) present (see for example the poor performance of samples 25 and 26, even though antibacterial cations are present).

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Thus, when antibacterial cations such as chlorhexidine are present in the aqueous phase, it is preferable to add the oil-soluble components to the oil phase. Such components include flavors, antioxidants (e.g. Vitamin E, BHA, BHTA), and essential oils which have antibacterial activity, e.g. eucalyptis oil, cinnamon oil.

On the other hand, samples in which the essential oil (peppermint) was solubilized in the aqueous phase using Tween 20 * (samples 25 and 26) were not effective, even though either 0.1% chlorhexidine (sample 25) or a combination of 0.05% of both cetylpyridinium chloride and chlorhexidine (sample 26) were present.

Bad breath is known to be chiefly due to microbial activity in the oral cavity. Sample 23A was tested against a leading commercial mouthwash (Listermint, Warner-Lambert Health Care Ltd., Eastleigh, Hants, England) for its ability to reduce bad breath levels, as determined by oral sulphide levels, using a sulphide monitor, (Interscan Model 1170, Interscan Ltd., Chatsworth, CA). One-quarter inch plastic straws are inserted into the sample inlet and the volunteer allows the end of the straw to enter the oral cavity, and breathes through his nose. The peak and steady-state value are recorded in parts per billion sulphide equivalents. Volunteers swished with either mouthwash for two thirty second periods at bedtime and tested peak and steady-state sulphide levels immediately upon awakening. Sample 23A reduced peak levels by 74%, and steady-state

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levels by 70%, as compared to no-mouthwash controls; in contrast, Listermint reduced peak levels by 51% and steady state levels by 45% as compared to no-mouthwash controls. Moreover, in a comparison of the ability of sample 23A to remove bound microorganisms from a solid surface, in an experiment analogous to that described in Table 3, sample 23A was superior to a wide variety of commercial mouthwashes in its ability to desorb bound cells.

As an additional example, we have tested formulations whose oil phase contains a combination of olive oil, peppermint oil and eucalyptus oil. One formulation, designated ODEX, has the following composition:

<u>Aqueous phase:</u>	
cetylpyridinium chloride	0.05%(w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.75% (w/w)
<u>Oil phase:</u>	
olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

Another formulation, designated ODEX+ has the following composition:

Aqueous phase:	
cetylpyridinium chloride	0.05% (w/w)
chlorhexidine digluconate	0.05% (w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.70% (w/w)
Oil phase:	
olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

The ability of these formulations to desorb microorganisms from polystyrene was tested as described in reference to Table 3. ODEX and ODEX+ removed 98% and 92%, respectively, of the bound RAG-1 bacteria.

Moreover, ODEX was tested in its ability to reduce bad breath, as compared to the following controls: (i) a commercial mouthrinse, Cepadont; (ii) a control in which the oil phase was entirely omitted; (iii) a control in which the olive and peppermint oil were omitted, and the eucalyptus oil solubilized in 6% Tween 20;* and (iv) no mouthrinsing control. Peak morning odor was measured using the Interscan 1170 sulphide analyzer. Two volunteers rinsed, prior to

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bedtime, for two consecutive 30 second periods. Immediately upon awakening, peak sulphide was measured. The results are summed up in Table 5 below:

Table 5: Overnight Effect of ODEX on Bad Breath

FORMULATION TESTED	Parts per billion sulphide equivalents	
	Volunteer 1	Volunteer 2
ODEX	25	20
(i) Cepadont	140	70
(ii) No oil phase	70	40
(iii) Solubilized eucalyptus oil	60	-
(iv) No mouthrinsing	117	150

These data show that the combination of aqueous phase and non-emulsified oil phase containing eucalyptus oil was more effective than any of the controls.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be

considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

WHAT IS CLAIMED IS:

1. A composition for desorbing bacteria from solid surfaces and from living tissues, which is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime said composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase; and

c) about 0.003 to about 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

2. A composition for desorbing bacteria from solid surfaces and from living tissues, according to claim 1 wherein said amphipathic cationic moiety is selected from pyridinium core surface-active cationic molecules, chlorhexidines, monalkyl quaternary ammonium compounds, dialkyl quaternary ammonium compounds, quaterniums and polyquaterniums, amine fluorides, cationic polysaccharides, and cationic polypeptides.

3. A composition for desorbing bacteria from solid surfaces and from living tissues, according to claim 1 wherein said amphipathic

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cationic moiety is selected from cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme.

4. A composition according to claim 1, where the oily phase is selected from olive oil, corn oil, coconut oil, soybean oil, safflower oil, octane, decane, tetradecane, hexadecane, white mineral oil or a mixture of any of these.

5. A composition according to claim 1 wherein said amphipathic cationic moiety is a chlorhexidine compound.

6. A composition according to claim 1 wherein said amphipathic cationic moiety is chitosan.

7. A mouthwash according to claim 1 comprising about 0.003 to about 0.5 w/w o an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

8. A composition according to claim 1 containing up to 0.3% of inorganic salts.

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9. A composition according to claim 1 which contains about 0.005 to 0.5 % cetylpyridinium chloride.

10. A composition according to claim 1 which contains about 0.005 to 0.5% cetylpyridinium chloride and about 0.005 to 0.5% of a chlorhexidine compound.

11. A composition according to claim 1 further comprising a pharmacologically acceptable dye which binds to microorganisms and debris in the presence of the other components.

12. A composition as claimed in claim 1 wherein said water immiscible oily phase comprises a member selected from the group consisting of a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydro carbon and mixtures thereof.

13. A compound as claimed in claim 8 wherein said inorganic salts are selected from the group consisting of phosphates, sodium chloride, magnesium chloride and fluorides.

14. A method for enhancing microbial adhesion at oil-water interface comprising admixing an aqueous microbial suspension and oil in the presence of an emphipathic cationic moiety in a composition as claimed in claim 1.

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חוק הפטנטים, התשכ"ז -- 1967
PATENTS LAW, 5727-1967

בקשה לפטנט
Application for Patent

C:23801

לשימוש הלשכה For Office Use	
מספר: Number	111554/4
תאריך: Date	8-11-1994
הוקדם/נדחה Ante/Post-dates	

אני, (שם המבקש, מענו -- ולגבי גוף מאוגד -- מקום התאגדותו)
I (Name and address of applicant, and, in case of body corporate-place of incorporation)

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מל רוזנברג
רחוב סמדר 34
רמת גן

ששמו Assignment
r, by virtue of

בעל אמצאה מכח העברה
of an invention, the title of which is:

שיטות ותרכובות לטיפול בשיער
(בעברית)
(Hebrew)

METHODS AND COMPOSITIONS FOR HAIR TREATMENT
(באנגלית)
(English)

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מבקש בזאת כי ינתן לי עליה פטנט

*בקשה חלוקה tion for Division	*בקשת פטנט מוסף - Application for Patent of Addition	*דרישה דין קדימה Priority Claim		
מבקשת פט Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
מס.	No. מס.			
מיום	dated מיום			

*יפוי כח: כללי/מיוחד - רצוף בזה / יוגש בעתיד
l / Individual - attached / to be filed later -
הוגש בענין

המען למסירת הודעות ומסמכים
Address for Service in Israel
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חתימת המבקש
Signature of Applicant
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111554/4

שיטות ותרכובות לטיפול בשיער

METHODS AND COMPOSITIONS FOR HAIR TREATMENT

MEL ROSENBERG
C: 23801

מל רוזנברג

METHODS AND COMPOSITIONS FOR HAIR TREATMENT

ABSTRACT OF THE DISCLOSURE

The invention provides methods and compositions for general treatment, cleaning and conditioning of head and body hair in man and animals, including but not limited to removal or cidal action against insects, particularly lice. The abovementioned composition is in the form of a.two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime, the composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to 50% w/w of a water immiscible oily phase, comprising a single oily substance or mixture thereof; and

c) about 0.003% to 5% of an amphipathic cationic moiety in an effective amount to treat the hair, and to enable the formation of the temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to ten hours of the formation thereof.

The method consists of shaking the above composition to form a temporary emulsion, applying said temporary emulsion to the hair, and washing out said composition from the hair.

The present invention relates to novel compositions which treat, condition and clean head and body hair.

The main use contemplated for the compositions of the present invention is in hygiene, with particular reference to ridding the hair of insects, especially live lice and nits. Compositions of the invention can also be used in entirely different applications, where hair is to be treated for a variety of conditions, including preshampoo softening or cleaning, preventing and reversing of tangled hair, pretreatment prior to hair coloring, etc.

According to the present invention there is now provided a composition for general treatment, cleaning and conditioning of head and body hair in man and animals, including but not limited to removal or cidal action against insects, particularly lice. The abovementioned composition is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime, the composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to 50% w/w of a water immiscible oily phase, comprising a single oily substance or mixture thereof; and

c) about 0.003% to 5% of an amphipathic cationic moiety in an effective amount to treat the hair, and to enable the formation of the temporary oil-in-water emulsion which emulsion breaks down and separates completely, or almost completely, within a period of about 10 seconds to ten hours of the formation thereof.

Amongst oils, which can be used to form said water immiscible oily phase there may be mentioned vegetable oils such as olive oil, corn oil, coconut oil, soybean oil, safflower oil, sesame oil, and almond oil. There can be used mono-, di-, and triglycerides, including caprylic triglyceride and capric triglyceride. There can also be used a wide variety of petroleum-based oils such as kerosene, various mineral oils and paraffins, silicone-based oils, and oils consisting of esters, such as isopropylmyristate, octylpalmitate, including esters of fatty acids and fatty alcohols. There can also be incorporated essential oils such as oil of rosemary, tea tree oil, oil of sage, birch leaf extract, oil of arnica, and oil of chamomille, which have special effects on hair, including anti-lice activity.

Preferably said amphipathic cationic moiety is selected from pyridinium core surface-active cationic molecules such as cetylpyridinium chloride, laurylpyridinium chloride, etc.; from chlorhexidines such as chlorhexidine, its diacetate, chlorhexidine digluconate, chlorhexidine dihydrochloride; from monoalkyl quaternary ammonium compounds (quats) such as benzalkonium chloride, cetalkonium chloride and bromide, lauralkonium chloride and bromide, soytrimonium chloride, PEG-5 stearyl ammonium lactate; from dialkyl quaternary ammonium compounds (diquats) such as dilauryl dimonium chloride, dicetyl dimonium chloride and bromide, dequalinium chloride, soyamido propyl benzyldimonium chloride, quaterniums such as quaternium 15 and polyquaterniums, cetyltriethylammonium bromide, etc.; amine fluorides, from cationic polysacchaarides, such as chitosan and its derivatives; from cationic polypeptides, such as poly L-lysine, poly D-lysine, lysozyme.

In especially preferred embodiments of the present invention said amphipathic cationic moiety is selected from cetylpyridinium chloride, chlorhexidine, polyquaternium 2,7,10,22, or 26, cetrimonium chloride, or behentrimonium chloride, which may also have anti-lice properties.

It is known that oils, such as olive oil, sesame oil and various essential oils may have a beneficial effect on hair. In the case of hair lice, kerosene is often used as a removal and cidal aid, and oil of rosemary, or other essential oils, as repellents. However, in these instances the oil is added as a single phase. Essential oils are also present in most commercial hair shampoos and conditioners, but are solubilized in order to obtain a confluent single phase. For example, Magdassi, S. and coworkers describe a method of microencapsulating oil droplets (The 6th Israeli Conference on Surface and Colloid Science, Zichron-Yaakov, Israel, abstract book page 65) in order to develop stable oil-in-water emulsions for natural oils, in order to formulate single phase lice repellent formulations. The present formulations differs in providing a two-phase composition, which becomes a temporary emulsion upon shaking. Both phases can then be simultaneously be administered to the hair with very high efficacy.

While the invention will now be described in connection with certain preferred embodiments in the following examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples

which include preferred embodiments will to serve to illustrate the practive of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptural aspects of the invention.

The following experimental results illustrate the invention, both in regard to the specific formulations and also as regards the mechanism of action of the present invention as regards treatment, conditioning and cleansing of hair.

The compositions of the invention preferably comprise a small quantity of an amphipathic cationic moiety which is preferrably a surface-active agent which is able to form and emulsion of limited life time. The emulsion ought to be formed upon shaking of the container which contains the two separate phases, and it should preferably remain in emulsion form for at least about 20 seconds. Experiments have shown that compositions of the invention form temporary emulsions which separate almost completely after a period of about 10 seconds to about 10 hours, depending, inter alia, on the degree of shaking.

To demonstrate the principle of the invention, a two-phase mixture consisting of 0.05% cetylpyridinium chloride (w/v), 15% isopropylmyristate (w/v) and water to 100% was tested. The mixture was shaken briefly and applied to the hair, and subsequently rubbed in. The hair was then washed with ordinary shampoo. The result was considerable softening of the hair, with a greatly improved feel.

Further, a two-phase commercial mouthwash (Assuta™ regular and mint, SOAD Ltd., Petach Tiqva) containing separate water and oily phases in a 85:15 w/w ratio, with 0.05% cetylpyridinium chloride as an amphipathic cationic agent, was tested on two children suffering from hair lice. The mixture was shaken briefly and applied to the hair, and subsequently rubbed in. Following brief rinsing, ordinary shampoo was then applied and the hair again washed, and combed. In both cases, live lice and nits were successfully removed and did not reappear for at least a month after use. Furthermore, the hair was observed to be softer and much more manageable. Tangles were easily undone by combing. These observations and effects were directly due to the effect of the two:phase formulation, since shampooing with ordinary shampoo had no such effects. With respect to control of hair lice, it was deemed that this treatment was much superior to existing

commercial treatments which contain harmful ingredients and do not successfully remove and kill nits, nor prevent reinfestation for long periods of time.

Examples of several formulations are described below:

Example 1:

Aqueous phase:

cetylpyridinium chloride	0.05% w/v
FD&C blue no. 1	0.1% of a 1% w/v aqueous solution
water	84.85% w/v

Oil phase:

isopropylmyristate	13.5% w/v
oil of chamomille	0.5% w/v
oil of rosemary	0.5% w/v
oil of sage	0.5% w/v

Example 2:

Aqueous phase:

benzalkonium chloride	0.2% w/v
water	74.80% w/v

Oil phase:

octylpalmitate	23.5% w/v
oil of rosemary	1.48% w/v
Antioxidant (BHTA)	0.01% w/v
D&C green No. 6	0.01% w/v

Example 3:

Aqueous phase:

polyquaternium 7	0.1% w/v
water	69.90% w/v

Oil phase:

capric triglyceride	29.0% w/v
tea tree oil	1.0% w/v

Example 4:

Aqueous phase:

cetylpyridinium chloride	0.05% w/v
benzalkonium chloride	0.15% w/v
water	69.80% w/v

Oil phase:

capric triglyceride	29.0% w/v
tea tree oil	1.0% w/v

Other uses of the invention include, but are not limited to its use for killing and removing body lice, and lice in animals as well as man, as well as for dandruff removal.

CLAIMS

1. A method of treating hair, comprising:

(i) providing a two-phase preparation comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase as hereinbefore defined; and

c) about 0.003 to about 5% of an amphipathic cationic moiety or moieties as hereinbefore defined, in an effective amount to treat the hair, and to enable the formation of a temporary oil-in-water emulsion which emulsion breaks down and separates completely, or almost completely, within a period of about 10 seconds to ten hours of the formation thereof;

with the proviso that said preparation does not contain a non-emulsifying foam stabilizer;

(ii) mixing said preparation to form said temporary emulsion;

(iii) applying said temporary emulsion to the hair; and

(iv) washing out said composition from the hair.

2. A method of treating hair according to claim 1, wherein said amphipathic cationic moiety is cetylpyridinium chloride.

3. A method of treating hair according to claim 1, wherein said amphipathic cationic moiety is benzalkonium chloride.

4. A method of treating hair according to claim 1, wherein said amphipathic cationic moiety is polyquaternium 2, 7, 10, 22 or 26.

5. A method of treating hair according to claim 1, wherein said amphipathic cationic moiety is cetrimonium chloride.

6. A method of treating hair according to claim 1, wherein said amphipathic cationic moiety is benhentrimonium chloride.

7. A method of treating hair according to claim 1, wherein said amphipathic cationic moieties consist of an amphipathic agent effective against hair lice, in combination with an amphipathic agent effective in creating the temporary emulsion.

8. A method of treating hair according to claim 1, wherein said oily phase is selected from vegetable, essential, mineral, silicon-based oils, synthetic oil, oily esters, fatty alcohols, or a mixture of any of these.

9. A method of treating hair according to claim 1, wherein over 50% w/v of said oily phase comprises isopropylmyristate.

10. A method of treating hair according to claim 1, wherein over 50% w/v of said oily phase comprises octylpalmitate.

11. A method of treating hair according to claim 1, wherein over 50% w/v of said oily phase comprises capric triglyceride.

12. A method of treating hair according to claim 1, wherein over 50% w/v of said oily phase comprises caprylic triglyceride.

13. A method of treating hair according to claim 1, wherein over 50% w/v of said oily phase comprises mineral oil.

14. A method of treating hair according to claim 1, wherein said oily phase contains a plant extract or mixture thereof with reputed effectiveness against lice.

15. A method of treating hair according to claim 1, wherein said oily phase contains tea tree oil.

16. A method of treating hair according to claim 1, wherein said oily phase contains oil of sage.

17. A method of treating hair according to claim 1, wherein said oily phase contains oil of rosemary.

18. A method of treating hair according to claim 1, wherein said oily phase contains oil of birch leaf.

19. A method of treating hair according to claim 1, wherein said oily phase contains oil of arnica.

20. A method of treating hair according to claim 1, wherein said oily phase contains oil of chamomile.

21. A method of treating hair according to claim 1, wherein the two-phase preparation is presented in a single container which is mixed before use to create a temporary emulsion.

22. A method of treating hair according to claim 1, wherein the two-phase preparation contains a pharmaceutically acceptable dye or dyes in the aqueous phase alone, the oily phase alone, or both.

23. A method of treating hair according to claim 1, such treatment being for the purpose of removing, killing and repelling lice and lice eggs (nits).

24. A method of treating hair according to claim 1, such treatment being for the purpose of conditioning hair.

25. A method of treating hair according to claim 1, such treatment being intended to kill, remove and repel insects, including body lice and nits, in human and animal hair and fur, as well as for dandruff removal.

26. A method of treating human hair according to claim 1.

27. A method of treating animal fur according to claim 1.

28. A composition for the treatment of hair comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase as hereinbefore defined; and

c) about 0.003 to about 5% of an amphipathic cationic moiety or moieties as hereinbefore defined, in an effective amount to treat the hair, and to enable the formation of a temporary oil-in-water emulsion which emulsion breaks down and separates completely, or almost completely, within a period of about 10 seconds to ten hours of the formation thereof;

with the proviso that said preparation does not contain a non-emulsifying foam stabilizer.

29. A method for the treatment of bodyhair in man and animals, which comprises mixing the composition according to claim 29 and applying it to the hair.

For the Applicant

Sanford T. Colb & Co.
Advocates and Patent Attorneys

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The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S. filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extension.

Commissioner of Patents and Trademarks

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NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

US006027081A

United States Patent [19]

Rosenberg

[11] Patent Number: 6,027,081

[45] Date of Patent: Feb. 22, 2000

[54] **ARTICLE HOLDER**

[75] Inventor: **Melvyn Rosenberg**, Ramat Gan, Israel

[73] Assignee: **Innoscent Ltd.**, Tel Hashomer, Israel

[21] Appl. No.: **09/013,024**

[22] Filed: **Jan. 26, 1998**

[51] **Int. Cl.**[7] **A47F 1/04**

[52] **U.S. Cl.** **248/37.6**; 211/65; 211/70.1; 248/300; 248/309.1

[58] **Field of Search** 248/213.2, 37.6, 248/300, 302, 309.1; 211/65, 70.1, 70.7

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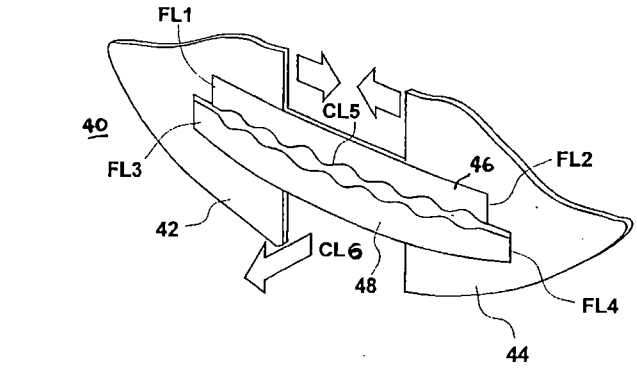
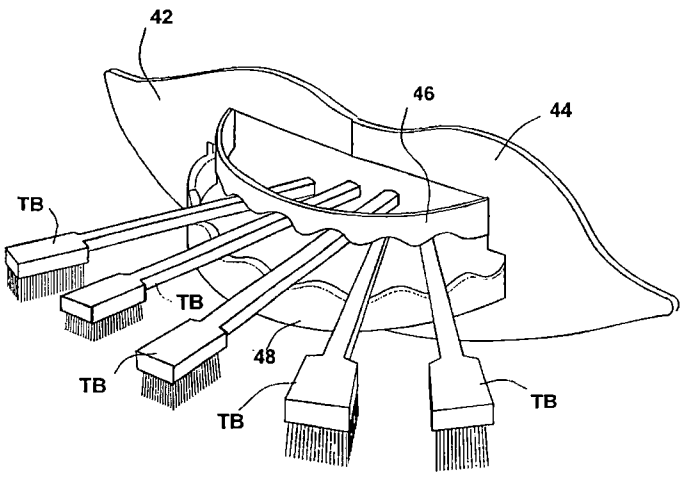
Primary Examiner—Derek J. Berger
Attorney, Agent, or Firm—Benjamin J. Barish

[57] **ABSTRACT**

A holder for an article of the type having a handle at one end and a working member at the opposite end, includes first and second fingers or arms extending in substantially parallel, spaced relation to each other to receive the article handle between them. One described embodiment is constructed as a flat blank of stiff sheet material for holding toothbrushes or similar articles. The flat blank is provided with cut and fold lines to define a pair of side sections normally spaced laterally apart, and a pair of strips separated from each other by cut lines and joined to the side sections by fold lines such that when the two side sections are moved toward each other to a mounting condition, the pair of strips become bowed outwardly of the side sections to serve as the upper and lower arms for supporting the articles between them.

8 Claims, 6 Drawing Sheets





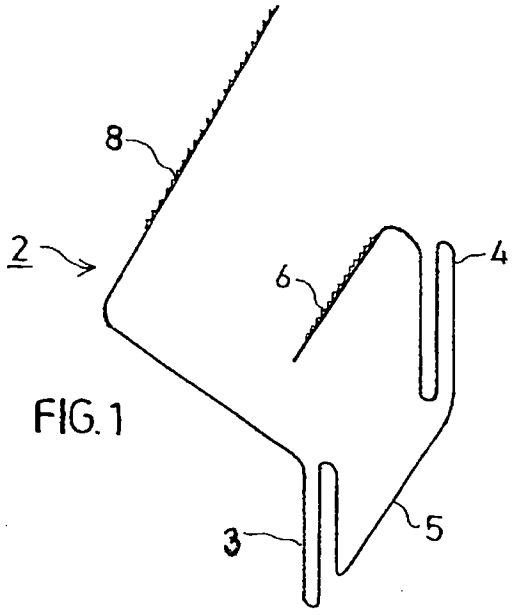


FIG. 1

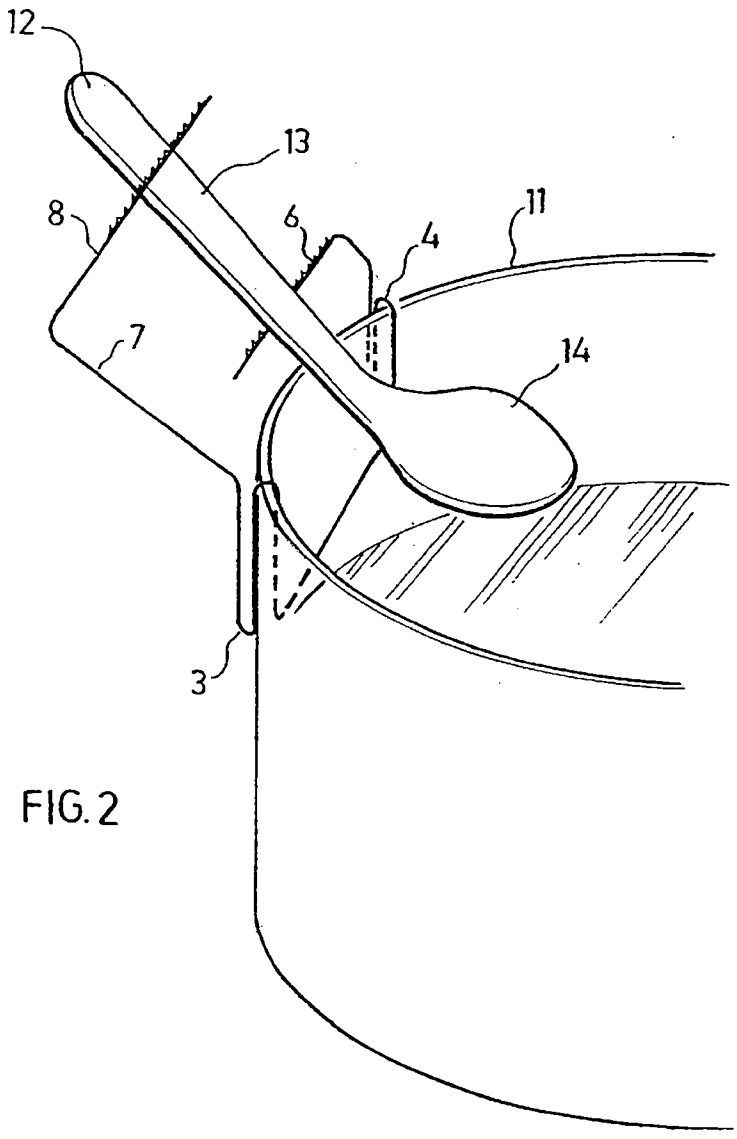
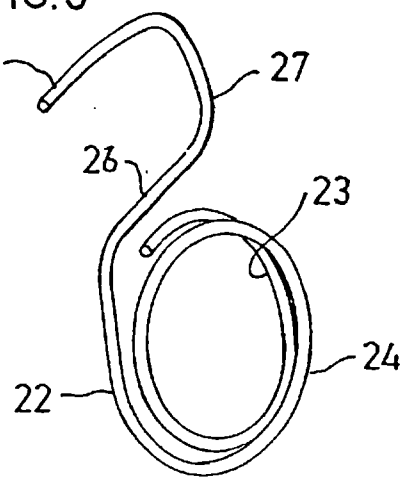


FIG. 2

FIG. 3



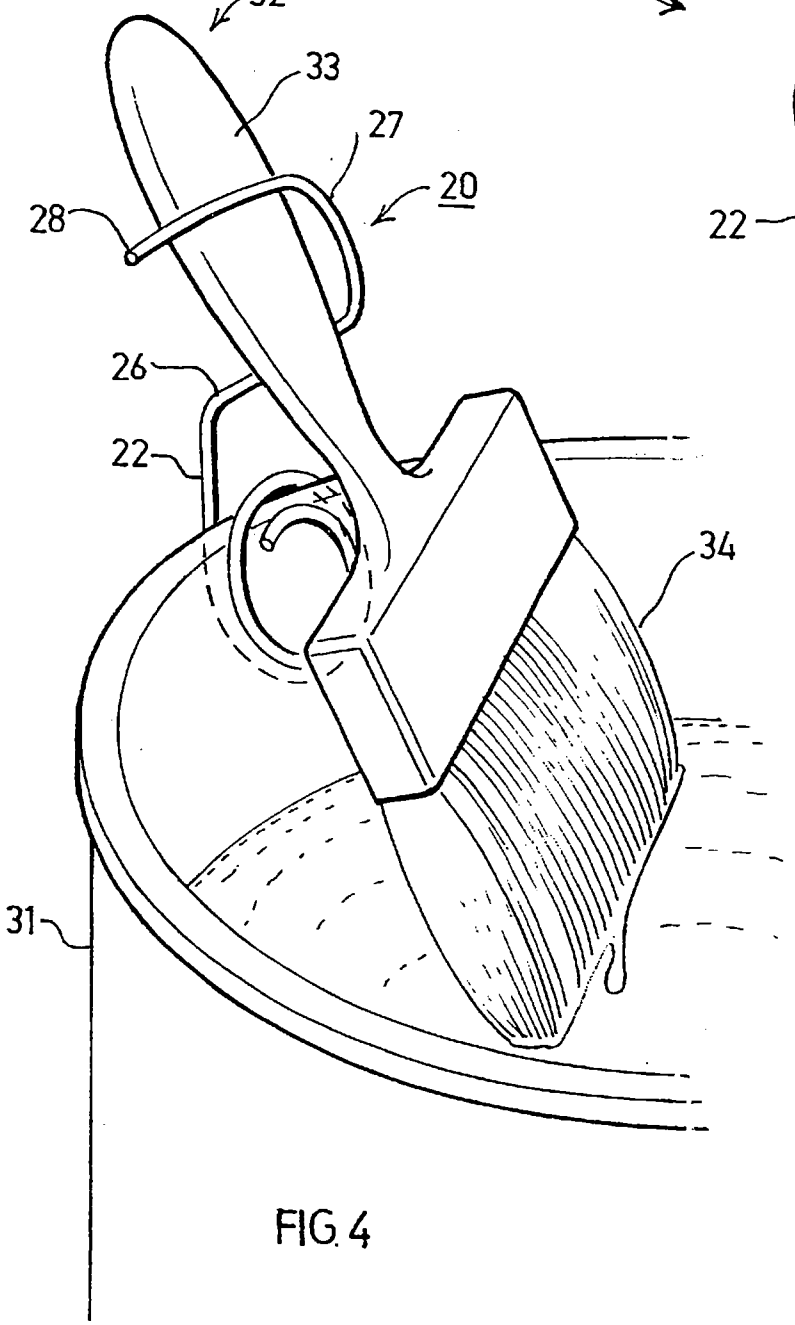


FIG. 4

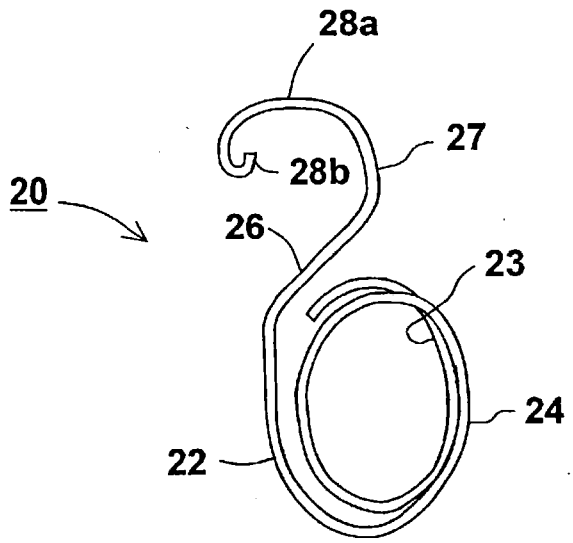


FIG. 5

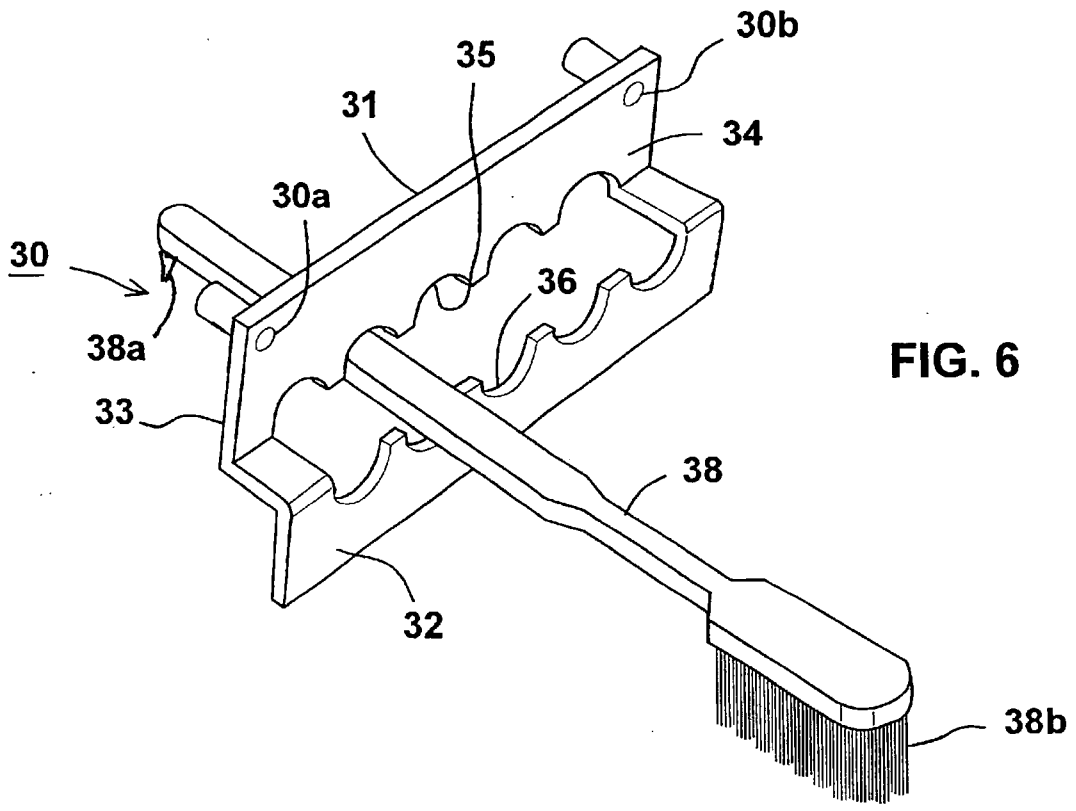


FIG. 6

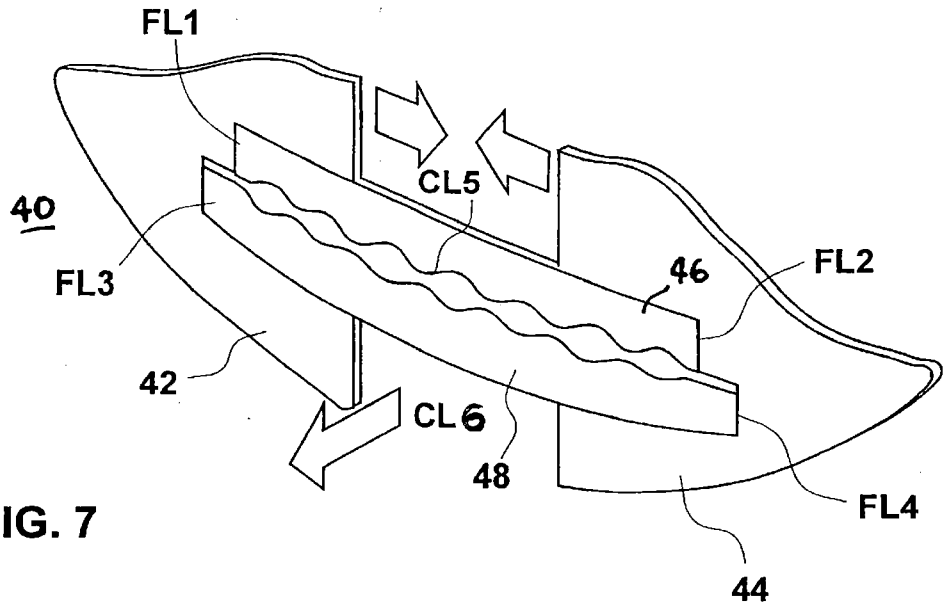


FIG. 7

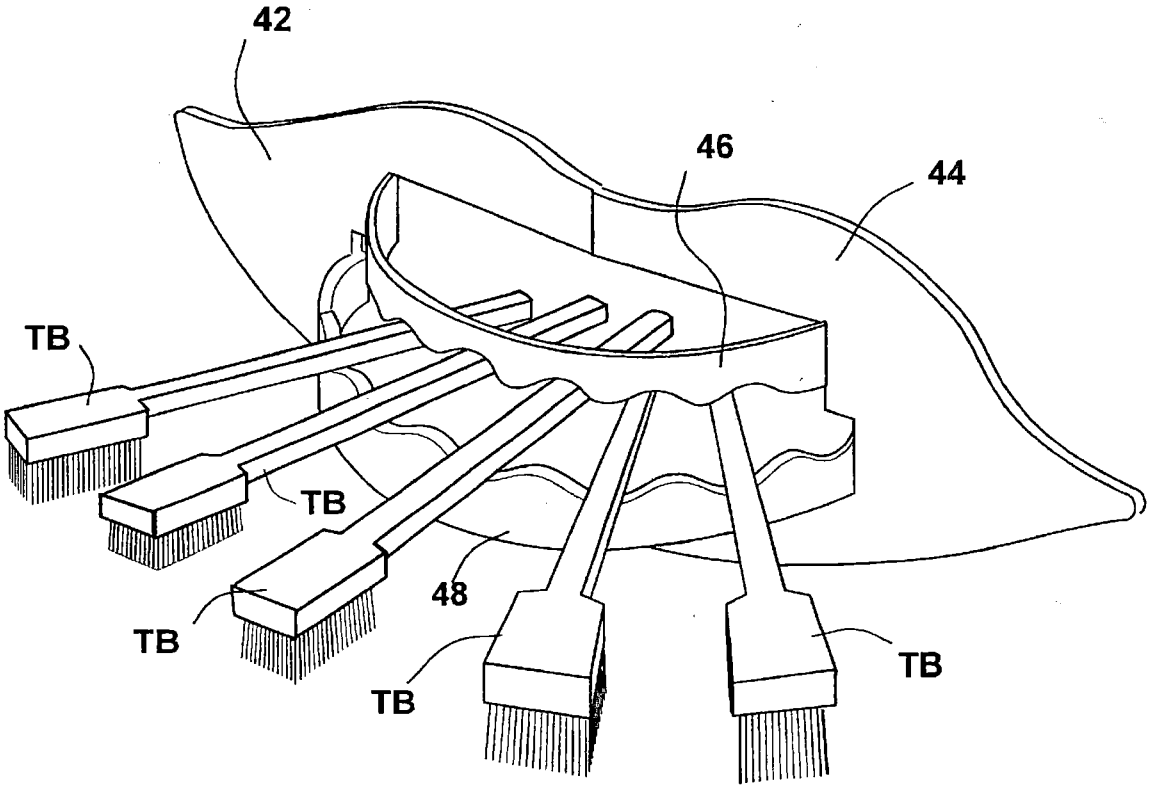
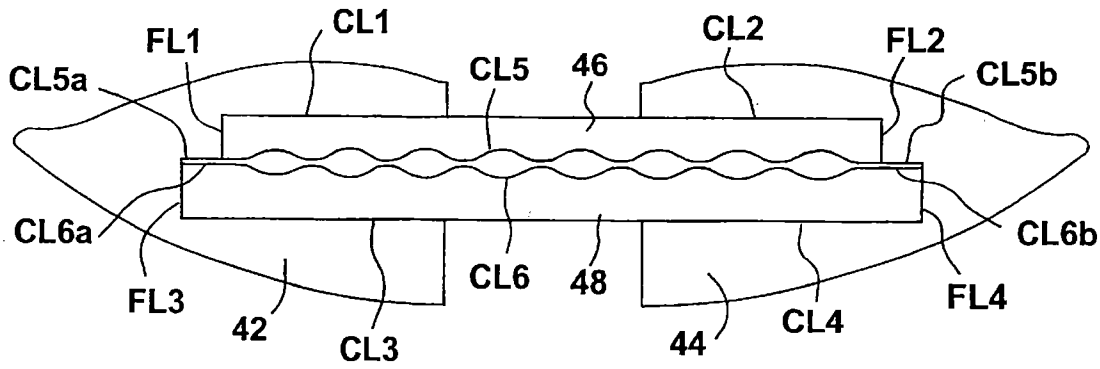


FIG. 8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CL1
CL5a
CL5
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CL2
FL2
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FL3
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CL3
CL6
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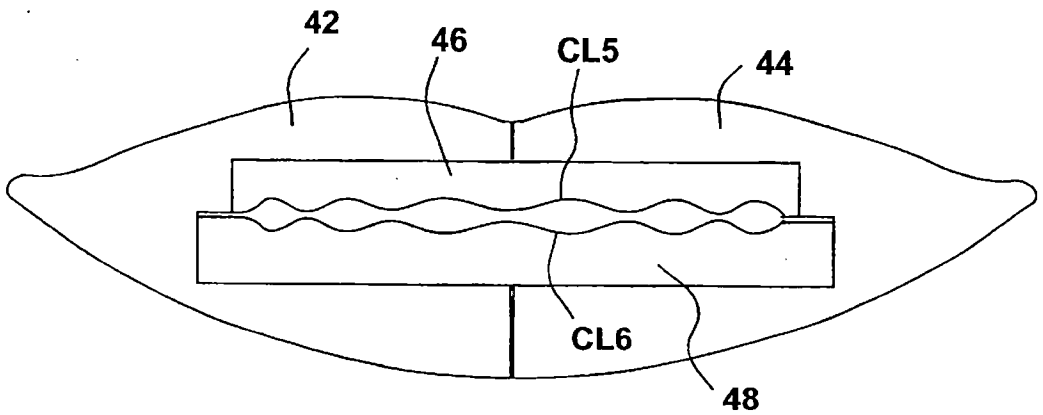
44
CL4
FL4

FIG. 9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CL5
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CL6
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FIG.10

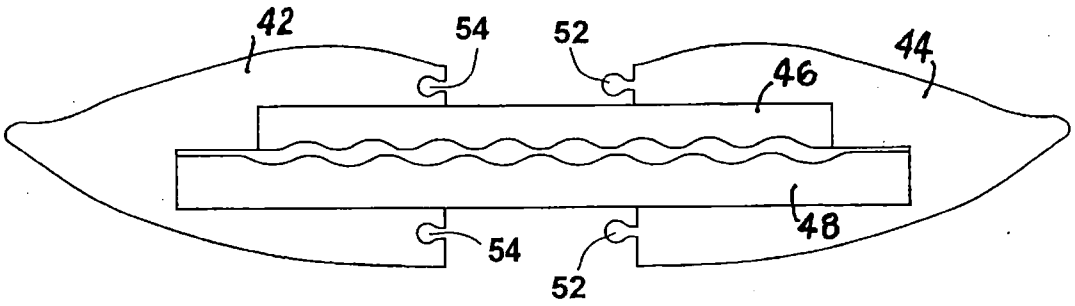

FIG. 11

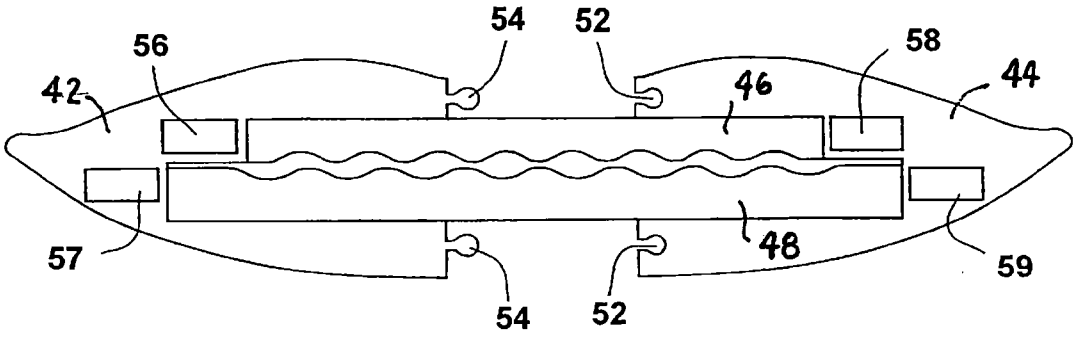

FIG. 12

ARTICLE HOLDER

FIELD OF THE INVENTION

The present invention relates to holders for conveniently holding various types of articles when not being used. The invention is particularly useful for holding articles such as stirring spoons, paint brushes, and particularly toothbrushes, and the invention is therefore described below with respect to such applications, but it will be appreciated that the invention could be used in many other applications as well.

BRIEF SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a flat blank of stiff sheet material for mounting to a vertical member to serve as an article holder for holding toothbrushes or similar articles, the flat blank being configured, and being provided with cut lines and fold lines, to define: a pair of side sections normally spaced laterally apart; and a pair of strips separated from each other by cut lines and joined to the side sections by fold lines such that when the two side sections of the flat blank are moved toward each other to a mounting condition for mounting the blank to the supporting member to serve as an article holder, the pair of strips become bowed outwardly of the side sections in the same direction for their complete lengths to said side sections and produce upper and lower arms for supporting the articles between them.

According to further features in the described preferred embodiment of this aspect of the invention, the cut lines and fold lines define, in the mounting condition of the blank, upper and lower bowed arms in which the lower bowed arm extends below is of a length longer than, and is spaced forwardly, of the upper bowed arm. Further, the pair of strips defining the bowed arms in the mounting condition of the blank are cut with cut lines of ripple configuration to define facing edges formed with a plurality of concave recesses tending to self-center the articles when engaging and supported by the bowed arms.

Article holder blanks constructed in accordance with the foregoing features can be produced in volume and at low cost because they merely require a simple stamping operation. Moreover, since the blanks are flat, they can be packaged in flat form, thereby requiring a minimum of space during storage, transportation, or other handling of the blanks before actual use as article holders.

It will be appreciated that the holder could be used for many other applications, such as for supporting various tools to a supporting member, etc.

Further features, advantages and applications of the invention will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. **1** illustrates one form of holder constructed in accordance with the present invention;

FIG. **2** illustrates one application of such a holder for holding a stirring spoon with respect to a cooking pot;

FIG. **3** illustrates a second form of holder constructed in accordance with the present invention;

FIG. **4** illustrates the holder of FIG. **3** used for holding a paint brush over a can of paint;

FIG. **5** illustrates a modification in the holder of FIGS. **3** and **4**;

FIG. **6** illustrates another holder construction in accordance with the present invention particularly useful for holding a plurality of toothbrushes;

FIG. **7** is a three-dimensional view illustrating another article holder constructed in accordance with the present invention, the article holder being shown in its initial flat condition;

FIG. **8** is a three-dimensional view illustrating the article holder of FIG. **7** in its mounting or deployed condition;

FIG. **9** is a front elevational view of the article holder blank in its original flat condition as shown in FIG. **7**;

FIG. **10** is a front elevational view of the article holder in its mounting condition as shown in FIG. **8**;

and FIGS. **11** and **12** are front and rear views, respectively, illustrating modifications in the construction of the blank of FIG. **7**.

DESCRIPTION OF PREFERRED EMBODIMENTS

The holder illustrated in FIGS. **1** and **2** of the drawings, and therein generally designated **2**, is of a one-piece construction made of wire bent into the specific configuration illustrated in the drawings. The wire may be of metal, e.g., 1.0–2.5 mm diameter stainless steel. It could also be of any suitable plastic material, or of metal coated with a plastic or ceramic material.

Holder **2** is bent to define an attaching section, constituted of two U-shaped bends **3**, **4**, joined together by a bridge **5**. one end of the one-piece wire unit terminates in a finger **6** integrally formed with the U-bend **4**; whereas the opposite end of the one-piece wire unit includes a leg **7** integrally formed with U-bend **3** and terminating in a second finger **8**.

As shown particularly in FIG. **2**, the mid-portion of the one-piece wire unit, including the two U-shaped bends **3**, **4** and the connecting bridge **5**, is applied over the rim of the cooking pot, therein designated **11** and is used for holding a stirring spoon **12** when the stirring spoon is not being used for stirring the food being cooked. Thus, when the one-piece wire unit is attached to the cooking pot **11**, the two fingers **6** and **8** constitute holding sections for holding the stirring spoon over the cooking pot. Thus, the two fingers **6** and **8** extend in spaced parallel relation to each other such that when handle **13** of the stirring spoon **12** is received between them, the upper surface of finger **6** engages the under surface of the inner end of handle **13**, and the weight of spoon **14** produces a rotary moment around finger **6** pressing the upper surface of the outer end of handle **13** firmly against the under surface of finger **8**, to hold the cooking spoon **12** between the two fingers.

It will also be seen that finger **8** is at a higher elevation than finger **6** when the holder is attached to the rim of the cooking pot **11**. The two fingers **6**, **8** will therefore hold the stirring spoon **12** at a declination in the inward direction of the cooking pot **11**, with the spoon end **14** overlying the interior of the receptacle. Thus, not only is the cooking spoon **12** held spaced from the cooking pot **11** so as not to be overheated by it, but also, it is held at a downward incline so that its drippings return back into the cooking pot. Moreover, holder **2**, even with the stirring spoon **12** held thereby, can not only be retained on the cooking pot **11** during the cooking time, but also allows the lid (not shown) to be placed on the cooking pot producing a slow venting of the food while it is being cooked.

Preferably, the bridge **5** between the two U-shaped bends **3**, **4**, of the holder **2** is sufficiently long and stiff to enable the

holder to be applied to cooking pots or other receptacles of different diameters. In addition, the two fingers **6**, **8** are preferably provided with outer surfaces of increased friction to prevent slippage of the stirring spoon **12**, or other article, held by the holder. Such increased friction may be produced by roughening the outer surfaces of the two fingers **6**, **8**, by providing them with a coating or sleeve of a plastic or ceramic material. Such a coating or sleeve also reduces the heat transfer from the cooking pot to the stirring spoon.

FIGS. **3** and **4** illustrate another holder, generally designated **20**, constructed in accordance with the present invention also in the form of a one-piece unit made of bent wire. Thus, holder **20** illustrated in FIGS. **3** and **4** includes an attaching section **22** in the form of two coaxial loops **23**, **24** adapted to receive between the mounting member, such as the rim of a container. Because of the elasticity of the wire used for making the holder **20**, the two loops **23**, **24** may be pushed apart sufficiently to slip the rim of the receptacle between them, thereby firmly retaining the holder so attached to the receptacle rim.

The attaching section **22** of holder **20** terminates in a U-shaped extension constituted of three legs **26**, **27** and **28**. Legs **26** and **28** are substantially parallel to each other and serve the function of the two holding sections, corresponding to holding fingers **6** and **8** in the holder of FIGS. **1** and **2**. They may be similarly provided with outer surfaces of increased friction to prevent slippage of the article held thereby.

FIG. **4** illustrates the holder **20** of FIG. **3** used for holding a paint brush **32** over a can of paint **31**. Thus, the holder is easily attached to the can of paint **31** by inserting the rim of the can between the two loops **23**, **24**, thereby enabling the two legs **26**, **28** to receive the handle **33** of the paint brush **32** between them and to be firmly retained in place by the weight of the paint brush **34**, while at the same permitting the paint to drain into the can.

FIG. **5** illustrates the holder of FIGS. **3** and **4** but of a slightly modified construction, in that leg **28** is provided with a concave recess **28***a* and terminates in a bead or lip **28***b*. Such a modification tends to self-center the handle of the article when applied to the holder and also to retain it in place.

FIG. **6** illustrates a further holder construction particularly useful for holding a plurality of toothbrushes. The holder illustrated in FIG. **6** can be constructed in the form of a bracket **30** to be attached to the wall via openings **30***a*, **30***b*. The holder includes a pair of holding sections or arms **31**, **32** extending in parallel spaced relationship, joined at their opposite sides by stepped bridges **33**, **34** integrally formed with the arms. Bridges **33** and **34** space the lower arm **32** in both the horizontal and vertical directions with respect to arm **31**, so that the lower edge **35** of arm **31** is located above and rearwardly of the upper edge **36** of arm **32**. In addition, the confronting edges **35**, **36** of the two arms **31** and **32** are formed with a series of concave recesses so as to receive a plurality of toothbrushes **38** in side-by-side relation.

It will thus be seen that each of the concave recesses in the confronting surfaces **35**, **36** of the two arms **31**, **32** is adapted to receive the handle of a toothbrush **38** and to stably support it with the opposite ends **38***a*, **38***b* of the toothbrush exposed to air. Such a holder is particularly useful with respect to toothbrushes provided at one end with a rubber pick, as shown at **38***a*, and a brush head **38***b* at the opposite end, since both ends are thus exposed to air. Also, the device minimizes dripping, and accommodates different sizes.

The article holder illustrated in FIGS. **7–10** of the drawings is particularly useful for holding a plurality of toothbrushes or similar type articles. FIG. **8** illustrates the article holder in its deployed condition wherein it assumes a three-dimensional configuration for supporting a plurality of toothbrushes. However, as shown in FIG. **7**, the article holder may be produced in the form of a flat blank **40**, thereby substantially reducing its manufacturing costs, and its volume when stored, shipped, and otherwise handled until its actual use.

As shown particularly in FIG. **9**, the blank **40**, in its initial flat condition, is constructed with a pair of side sections **42**, **44**, spaced laterally apart, and a pair of bridgeing strips **46**, **48**, extending between the two side sections. Strips **46**, **48** are adapted to form bowed arms, as shown particularly in FIG. **8**, when the two side section are moved towards each other into abutting contact at their flat facing edges, as shown in FIG. **10**, when the device is to be mounted at the point of use. For this purpose, the two strips **46**, **48**, are joined to the two side sections **42**, **44** of the blank, by a plurality of horizontal cut lines CL_1–CL_4 (FIG. **9**), and a plurality of vertical fold lines FL_1–FL_4. In addition, the two strips **46**, **48**, are separated from eachother by further horizontal cut lines CL_5, CL_6.

The latter horizontal cut line CL_5, CL_6 are formed with a ripple or scalloped configuration, to thereby define facing edges having a plurality of concave recesses tending to self-center the toothbrushes when supported by the article holder, as shown in FIG. **8**.

The upper strip **46** is cut from the two end section **42**, **44**, by horizontal cut lines CL_1, CL_2 at the upper edge of the opposite ends of the strip; and strip **46** is joined to the two end sections **42**, **44**, by vertical fold lines FL_1, FL_2 at the opposite ends of the strip. Similarly, the lower strip **48**, is cut from the two end sections **42**, **44** by horizontal cut lines CL_3, CL_4 at the opposite ends of the lower edge of the strip; and strip **48** is joined to the two end sections **42**, **44**, by vertical fold lines FL_3, FL_4 at the opposite ends of the strip.

As can be seen particularly in FIG. **9**, the two vertical fold lines FL_3, FL_4 at the lower end of strip **48** are spaced farther apart than the vertical fold lines FL_1, FL_2 at the opposite ends of strip **46**. As also seen in FIG. **9**, the end portions of the two horizontal cut lines CL_5, CL_6, between fold lines FL_1, FL_3 on one side, and fold lines FL_2 and FL_4 on the opposite side, are straight, i.e. non-rippled, as shown at CL_{5a}, CL_{5b} and CL_{6a}, CL_{6b}, respectively. With such a construction, when the flat blank **40** is deployed to its mounting condition by moving the two end section **42**, **44**, into abutting contact at their flat facing edges, as shown particularly in FIG. **8**, both strips **46** and **48** produce bowed arms, with the bowed arm of the lower strip **48** extending below and spaced forwardly of the bowed arm of the upper strip **46**.

Such an arrangement permits the toothbrushes TB to be conveniently applied with the brush head facing outwardly, as shown in FIG. **8**. The weight of the brush head produces a rotary movement causing the upper surface of the brush handle to be pressed against the lower surface of the upper arm **46**, and the lower surface of the brush handle to be pressed against the upper surface of arm **48**, thereby holding the toothbrush in a manner enabling it to be conveniently removed as desired. It will also be seen that the rippled facing edges of the two arms **6**, **8**, produce concave surfaces which tend to self-center the toothbrushes when so supported by the holder.

FIGS. **11** and **12** are front and rear views respectively, of a blank similar to that described above with respect to FIGS. **7–10**, but including the following modifications: The blank

is cut to define tongues **52** and complementary recesses **54** in the abutting edges of the side sections **42**, **44** to aid in retaining them in abutting contact when moved together as shown in FIG. **8**. Also, the rear faces of the side sections **42**, **44** are provided with adhesive strips **56**, **57**, **58**, **59**, each covered by a removable protective liner, for adhesively mounting the blank to a vertical supporting member (e.g., the wall) as shown in FIG. **8**.

While the invention has been described with respect to several preferred embodiments, it will be appreciated that these are set forth merely for purposes of example, and that many modifications may be made. For example, in the FIGS. **1** and **2**, holder the mid-section including the two U-bends **3**, **4**, and the bridge **5**, need not be stiff; also, they could be constructed as a separate unit, and the two fingers **6**, **8**, could be attached to them. Further, the unit could be constructed of a bent strip of plastic or metal, rather than of bent wire. Also, the holder, particularly that of FIG. **6**, could be constructed as a stand-alone device. In addition, the described holders could be designed for holding various working tools, writing implements, or the like to a wall-mounted bracket or to a stand-alone unit.

Further, in the embodiments illustrated in FIGS. **7–11**, the flat blank could be produced by injection molding plastic, as well as by stamping plastic, cardboard, etc. sheets. Also, instead of merely abutting the edges to the pair of side sections (**42**, **44**) as illustrated in FIGS. **7–10**, or providing tongue-and-recess retainers as illustrated in FIGS. **11**, **12**, the edges of the side sections could be made to overlap and to be retained by snap-on fasteners, adhesive, "Velcro" (Reg. TM) fastener strips, or other means. The embodiments of FIGS. **7–11** can be produced in volume and at low cost such as to enable them to be used as "give-away" items in promoting the sale of various products, such as toothbrushes, tooth paste, etc.

Many other variations, modifications and applications of the invention will be apparent.

What is claimed is:

1. A flat blank of stiff sheet material for mounting to a vertical member to serve as an article holder for holding articles, said flat blank being configured, and being provided with cut lines and fold lines, to define:

a pair of side sections spaced laterally apart;

and a pair of strips, constituting an upper strip and a lower strip, separated from each other by said cut lines and joined to said side sections by said fold lines such that when the two side sections of the flat blank are moved toward each other to a mounting condition for mounting the blank to the vertical member to serve as an article holder, the pair of strips become bowed outwardly of the side sections in the same direction for their complete lengths to said side sections and produce upper and lower bowed arms for supporting the articles between them;

wherein said cut lines and fold lines produce, in the mounting condition of the blank, said upper and lower bowed arms in which the lower bowed arm extends below, is of a longer length than, and is spaced forwardly of, the upper bowed arm.

2. The blank according to claim **1**, wherein said cut lines include cut lines of ripple configuration between the pair of strips producing the bowed arms in the mounting condition of the blank to define facing edges formed with a plurality of concave recesses tending to self-center the articles when engaging and supported by the bowed arms.

3. The blank according to claim **1**, wherein the blank is configured and cut to define flat facing edges in the pair of side sections to be brought into abutment with each other in the mounting conditon of the blank.

4. The blank according to claim **1**, wherein the blank is formed with:

first and second horizontal cut lines between the pair of side sections and upper edges of the opposite ends of the upper strip;

third and fourth horizontal cut lines between the pair of side sections and lower edges of the opposite ends of the lower strip;

fifth and sixth horizontal cut lines defining the lower edge of the upper strip and the upper edge of the lower strip, respectively;

first and second vertical fold lines at the opposite ends of the upper strip extending between the fifth horizontal cut line, and the first and second horizontal cut lines, respectively;

and third and fourth vertical fold lines at the opposite ends of the lower strip extending between the sixth horizontal cut line and the third and fourth horizontal cut lines, respectively.

5. The blank according to claim **4**, wherein said third and fourth vertical fold lines are spaced farther apart from the first and second vertical fold lines such that, in the mounting condition of the blank, the pair of strips define said upper and lower bowed arms in which the lower bowed arm extends below and is spaced forwardly of the upper bowed arm.

6. The blank according to claim **4**, wherein said fifth and sixth horizontal cut lines are of ripple configuration such that, in the mounting condition of the blank, said ripple lines define concave recesses between the lower edge of the upper bowed arm and the upper edge of the lower bowed arm tending to self-center the articles when supported by said arms.

7. The blank according to claim **1**, wherein the blank is cut to define tongues and complementary recesses in the pair of side sections to aid in retaining the side sections when moved towards each other in the mounting conditon of the blank.

8. The blank according to claim **1**, wherein the pair of side sections have rear faces provided with adhesive strips for adhesively mounting the blank to the vertical member.

* * * * *

File: 2002-us-patent-6439435.pdf

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S. filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extensions.

Director of the United States Patent and Trademark Office

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NOTICE

If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number of timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the United States Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

US006439435B2

(12) **United States Patent**
Rosenberg

(10) **Patent No.: US 6,439,435 B2**
(45) **Date of Patent: *Aug. 27, 2002**

(54) **DEVICE AND METHOD FOR DEODORIZING SHOES**

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(73) Assignee: **Innoscent Ltd.**, Tel Hashomer (IL)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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§ 371 (c)(1),
(2), (4) Date: **Aug. 19, 1999**

(87) PCT Pub. No.: **WO98/38881**

PCT Pub. Date: **Sep. 11, 1998**

(30) **Foreign Application Priority Data**

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(51) **Int. Cl.**[7] **B67D 5/64**; B67D 5/06; B65D 88/54; B65D 83/00

(52) **U.S. Cl.** **222/162**; 251/185.1; 251/330; 251/402.19

(58) **Field of Search** 222/162, 185.1, 222/394, 497, 173, 184, 174, 402.1, 402.19, 330

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(57) **ABSTRACT**

A deodorizer device for deodorizing shoes, includes a container containing deodorizer material, and a dispenser assembly at one end of the container for dispensing deodorizer material. The dispenser assembly includes a feed tube having one end located within the container and the opposite end communicating with one or more discharge openings for discharging deodorizer material laterally of the container. The dispenser assembly further includes an actuator attached at one end to the feed tube and configured to enable the actuator to be inserted into a shoe, when the container is inverted, to stably support the container in the inverted position, and to be pressed against the inner surface of the shoe bottom for discharging a quantity of deodorizer material from the discharge opening laterally into the shoe.

16 Claims, 2 Drawing Sheets

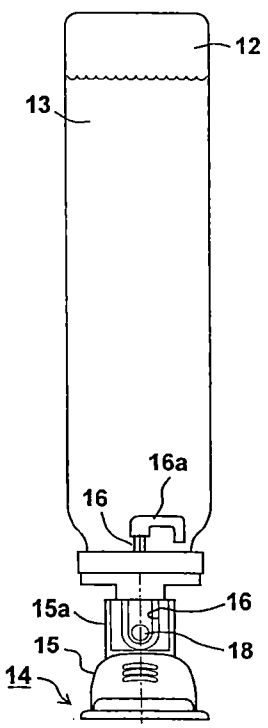
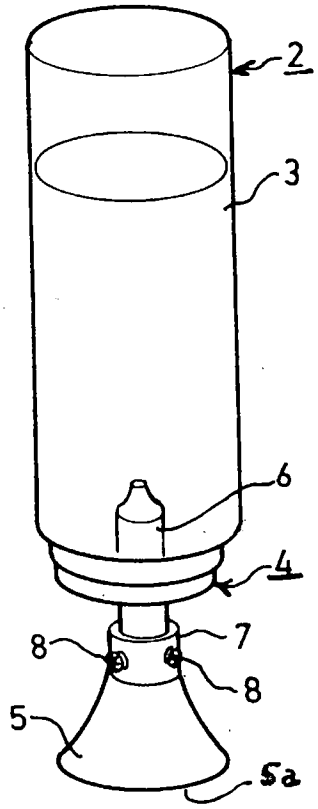


FIG 1



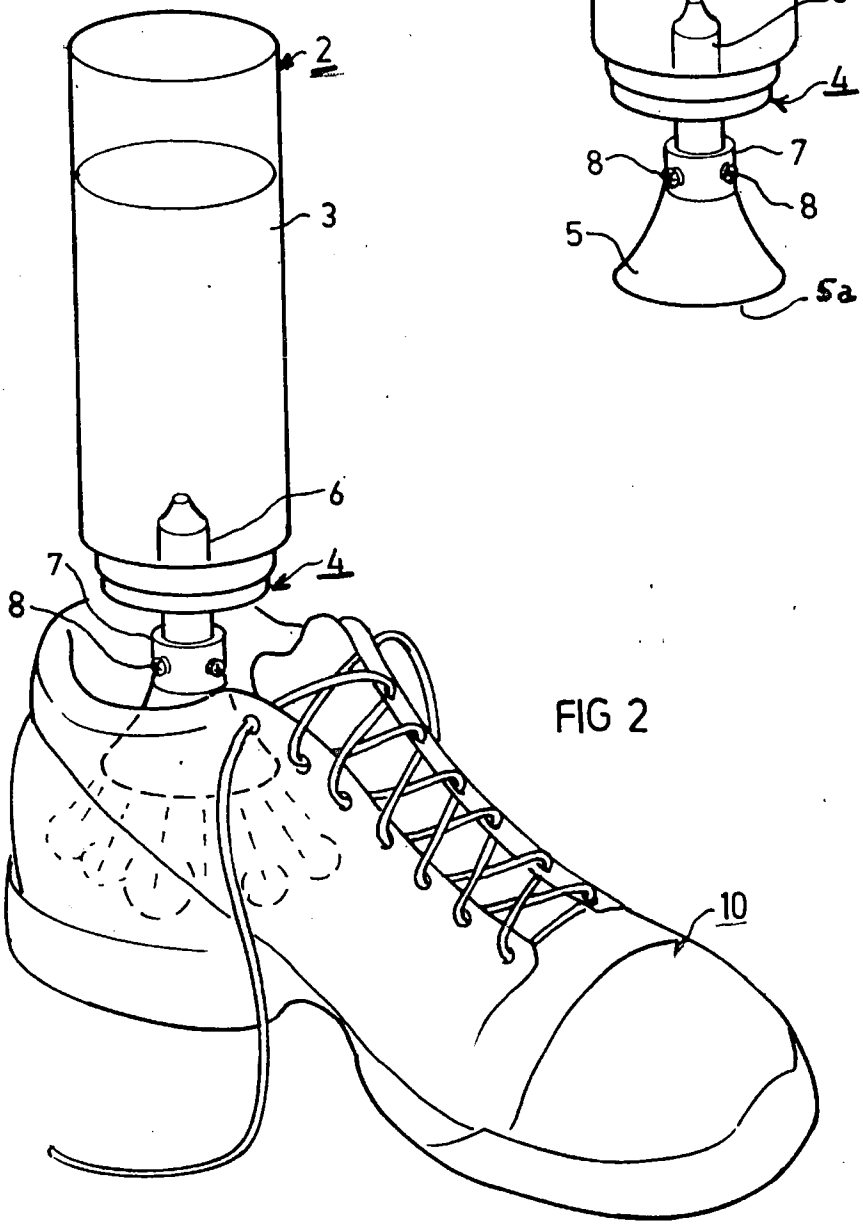


FIG 2

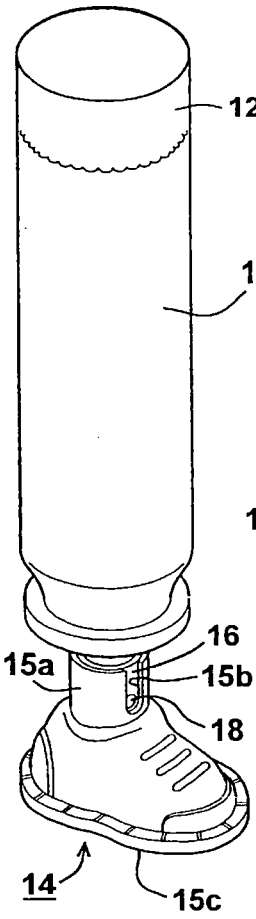


FIG. 3

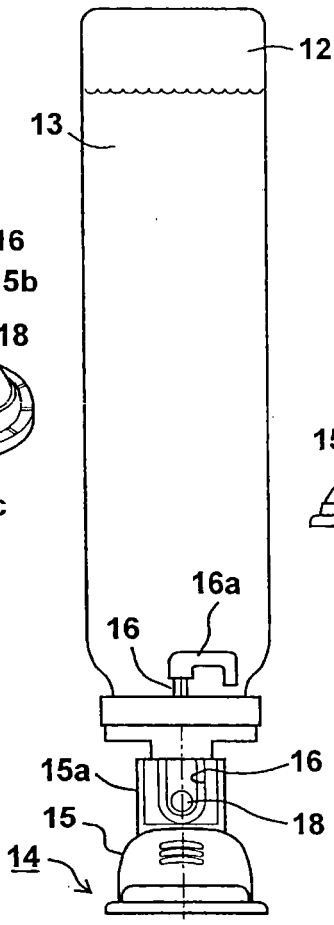


FIG. 4

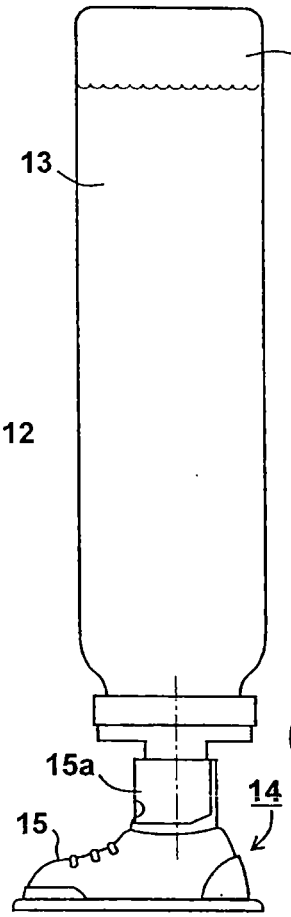


FIG. 5

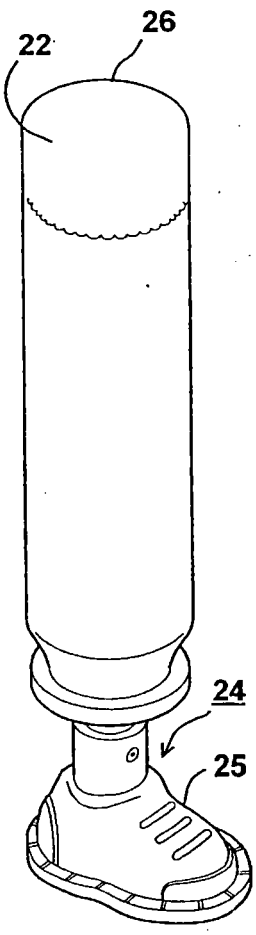


FIG. 6

DEVICE AND METHOD FOR DEODORIZING SHOES

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to deodorizer devices and methods, and particularly to devices and methods for deodorizing shoes.

Shoe odor is a common problem. For this reason, it is generally recommended not to wear the same pair of shoes for extended periods of time in order to permit them to air out by themselves. However, this is not always possible or convenient. Therefore, dispensers have been developed for dispensing deodorizer materials directly into the shoes, but such dispensers dispense the deodorizer material from the top of the dispenser. This is somewhat awkward since the dispenser must be held by one hand and the shoe held by the other, and the user must manipulate the dispenser and/or the shoe in order to dispense the material within the shoe. Moreover, with the conventional shoe deodorant sprays, it is almost impossible to spray into the foot portion of high boots or high top sneakers.

OBJECTS AND BRIEF SUMMARY OF THE INVENTION

An object of the present invention is to provide a device and method having advantages in the above respects for deodorizing shoes.

According to one aspect of the present invention, there is provided a deodorizer device for deodorizing shoe, comprising: a container containing deodorizer material; and a dispenser assembly at one end of the container for dispensing deodorizer material therefrom; the dispenser assembly including a feed tube having one end located within the container and the opposite end communicating with at least one discharge opening externally of the container for discharging deodorizer material laterally of the container; the dispenser assembly further including an actuator attached at one end to the feed tube; the actuator being in the form of a cap having a socket at one end coaxial with, and dimensioned to receive, the opposite end of the feed tube, the opposite end of the cap being dimensioned and configured to enable the container to stably rest in the inverted position within the shoe with the one end of the container and the dispensing assembly disposed within the shoe, and the opposite end of the container extending outwardly of the shoe, to enable a quantity of the deodorizer material to be dispensed into the shoe by pressing against the opposite end of the container.

The present invention thus distinguishes from conventional shoe sprays, which have the dispensing valve located on the top of the container and are displayed in stores, kept by the consumer, and used by the consumer, in this upright position. A device constructed in accordance with this feature of the invention would have the dispensing valve located on the bottom of the container, and would be displayed, stored and used with the dispensing valve on the bottom. This means the package should be stable when standing in this inverted position. By having the package stable in this inverted position, the user can easily pick it up, place it directly into a shoe (without inverting it), and just press down to actuate the valve. With conventional shoe sprays the valve is actuated by a finger pressing against the actuator button, whereas in the novel device, the valve is actuated by the shoe pressing against the actuator.

According to a further aspect of the present invention, there is provided a method of deodorizing shoes, by providing a deodorizer device as described above; inserting the deodorizer device into a shoe, with the container in inverted position such that the actuator is at the bottom of the container; stably resting the container in this inverted position on the inner surface of the shoe bottom; and pressing downwardly the opposite end of the container for discharging a quantity of deodorizer material into the shoe.

The invention may be embodied in different types of dispensers. For example, the container may be of the type which contains a quantity of a deodorizer material pressurized by a gas propellant, in which case the dispenser assembly would include a normally-closed valve which is opened by the actuator when pressed against the inner surface of the shoe bottom to dispense a quantity of deodorizer material via the discharge opening laterally of the dispenser assembly and into the shoe. Another type is one wherein the container contains a quantity of an unpressurized deodorizer material (liquid or powder), and the dispenser assembly includes a manual pump which is pumped by the actuator when pressed against the inner surface of the shoe bottom to dispense a quantity of the deodorizer material via the discharge opening laterally of the dispenser assembly and into the shoe.

Further features and advantages of the invention will be apparent from the description below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. **1** illustrates one form of deodorizer device constructed in accordance with the present invention;

FIG. **2** illustrates the deodorizer device as applied to a shoe for deodorizing the shoe;

FIGS. **3**, **4** and **5** are perspective, front and side views respectively, of another deodorizer device constructed in accordance with the present invention; and

FIG. **6** is a perspective view illustrating a further deodorizer device constructed in accordance with the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

With reference to FIG. **1**, there is illustrated a deodorizer device comprising a container **2** for containing a quantity of a liquid deodorizer material **3**. A dispenser assembly, generally designated **4**, is attached to the container **2** so as to be at the bottom of the container, rather than at the top as in a conventional dispenser of this type. Dispenser assembly **4** includes an actuator **5** in the form of a cap which is engageable with the inner surface of the bottom of the shoe, as illustrated in FIG. **2**, for dispensing the deodorizer material **3**. Dispenser assembly **4** further includes a feed tube **6** leading into the interior of container **2**, and a socket **7** in the form of a collar formed at one end of cap **5**. Socket **7** is coaxial with, and is dimensioned to receive, the external part of feed tube **6** and has one or more discharge openings **8** around its circumference. Each of the discharge openings **8** communicates via feed tube **6** with the interior of container **2**.

As shown in FIG. **1**, the opposite end **5***a* of actuator cap **5**, which engages the inner surface of the shoe bottom as shown in FIG. **2**, is of large cross-sectional area, the cross-sectional area of the actuator decreasing towards collar **7** formed with one or more discharge openings **8**. Preferably, actuator cap **5** is of a generally conical configuration.

FIG. **2** illustrates the manner of using the deodorizer device for deodorizing a shoe, generally designated **10**. Thus, the deodorizer device is inserted into the shoe, with the container **2** in the illustrated inverted position so that the actuator cap **5** is at the bottom of the container, and the outer surface of end **5***a* of the actuator cap **5** is pressed against the inner surface of the shoe bottom. This depression of the actuator cap **5** actuates the dispenser assembly **4** to discharge deodorizer material **3** via the discharge openings **8** around the circumference of the dispenser assembly **4** and in all directions into the interior of the shoe **10**.

As one example, the container **2** could contain a quantity of liquid deodorizer material **3** pressurized by a gas propellant; and the dispenser assembly **4** could include a normally closed valve which is opened by actuator cap **5** when pressed against the shoe bottom to dispense a quantity of the deodorizer material **3** via the discharge openings **8** around the dispenser assembly **4** and in all directions into the interior of the shoe **10**. Any known deodorizer material could be used, e.g., in the form of an anhydrous suspension, a suspension in water or some other liquid carrier, or dry powder.

As another example for implementing the invention, the container **2** could contain a quantity of an unpressurized liquid deodorizer material **3**, and the dispenser assembly **4** could include a manual pump which is pumped by actuator cap **5** when pressed against the inner surface of the shoe bottom to dispense a quantity of the deodorizer material via the discharge openings **8**.

The conical shape of actuator cap **5** provides a large surface area at its end **5***a* for engagement with the shoe bottom, and a relatively smaller annular area occupied by the discharge openings **8** for discharging the deodorizer material in all directions. In addition, this configuration of the actuator enables container **2** to stably rest in the inverted position within the shoe. It will be appreciated, however, that the actuator cap **5** may take other configurations.

Following is one example of a deodorizer material formulation that can be used:

ethanol	70%
water	23.0%
preventol R80	3.2%
NP10	2%
orange terpenes	1.0%
pine oil	0.5%
perfume	0.15%
citric acid	0.1%
EDTA	0.05%

FIGS. **3–5** illustrate another deodorizer device constructed in accordance with the invention, comprising a container **12** for containing a quantity of liquid deodorizer material **13**, and a dispenser assembly **14** attached to the container **12** so as to be at the bottom of the container. Dispenser assembly **14** includes an actuator cap **15** which is also engagable with the inner surface of the bottom of the shoe for dispensing the deodorizer material **13**, and a feed tube **16** leading into the interior of container **12**. In this case, however, feed tube **16** is integrally formed with a u-shaped extension **16***a* (FIG. **4**) such that the open end **16***b* of the feed tube **16** is at the bottom of container **12** when in its inverted position to enable the container to dispense substantially all its contents when in the inverted position.

In the modification illustrated in FIGS. **3** and **4**, only a single discharge opening **18** is formed in feed tube **16**. In addition, the actuator cap **15** is in the form of a shoe. Thus, one end of the cap **15** is formed with a socket **15***a* coaxial with and dimensioned for receiving the external end of feed tube **6**, and with a slot **15***b* for exposing the discharge opening **18** in the feed tube. The opposite bottom end **15***c* of actuator cap **15** is formed with an enlarged surface area for engaging the upper surface of the bottom of the shoe (**10**, FIG. **2**), when the deodorizer device is inserted in inverted position within the shoe as shown in FIG. **2**.

The bottom end **15***c* of the actuator cap **15** is preferably of sufficiently large surface so as to enable the container **12** to be stably supported in inverted position in the shoe such that, whenever a quantity of deodorizer material is to be dispensed, the user may merely press the opposite end of container **13** downwardly to discharge a quantity of the deodorizent material from discharge opening **18**. Discharge opening **18** is oriented to direct the discharged deodorizer material towards the tip of the shoe cap **15**, and of the shoe being deodorized (**10**, FIG. **2**).

In all other respects, the deodorizer device illustrated in FIGS. **3–5** is constructed, and is used in the same manner, as described above with respect to FIGS. **1** and **2**.

FIG. **6** illustrates a further construction similar to that of FIGS. **3–5**, except that the actuator cap **25**, also in the shape of a shoe for stably resting the container **22** in an inverted vertical position, is built into the dispenser assembly **24**, rather than being constructed as a separate cap applied to the container. Another modification is that the opposite end of the container **26** is of a dome-shaped configuration, which precludes supporting the container from that end. This construction thereby directs the user to apply the container in its inverted position. It also facilitates pressing the container downwardly, while stably resting in its inverted position within the shoe, to discharge a quantity of the deodorizer material into the shoe.

While the invention has been described with respect to two preferred embodiments, it will be appreciated that these are set forth merely for purposes of example, and that many other variations, modifications and applications of the invention may be made.

What is claimed is:

1. A device for deodorizing shoes, comprising:

a container containing a deodorizer material;

and a dispenser assembly at one dispensing end of the container for dispensing the deodorizer material therefrom;

said dispenser assembly including a feed tube having an inner region with one open end located within the container and an outer region with an opposite, dispensing end communicating with at least one discharge opening external of the container for discharging the deodorizer material laterally of the container;

said dispenser assembly further including an actuator attached at one end to said feed tube;

said actuator being in the form of a cap having a socket at a connecting end coaxial with, and dimensioned to receive said dispensing end of the feed tube and including a cylindrical wall formed with a slot for exposing the discharge opening with which the dispensing end of the feed tube communicates, the opposite, supporting end of said cap being dimensioned and configured to enable the container to stably rest in the inverted position within the shoe with said dispensing end of the container and said dispenser assembly disposed within the shoe, and the opposite end of the container extending outwardly of the shoe, to enable a quantity of the

deodorizer material to be dispensed into the shoe by pressing against said opposite end of the container,

wherein the deodorizer material is a liquid and the container additionally contains a pressurized gas,

wherein the inner portion of said feed tube is integrally formed with a u-shaped extension such that the open end of the feed tube is near the dispensing end of the container, thereby enabling dispensation of substantially all of the deodorizer material when the container is inverted,

wherein the dispenser assembly comprises an internal 360° dispensing valve capable of dispensing the deodorizer material when the container is inverted and which remains in a closed position until the actuator is pressed against the inner surface of a shoe bottom, and

wherein the discharge opening is oriented to discharge the deodorizer material towards a shoe tip.

2. A method of deodorizing shoes, comprising:

providing a deodorizer device according to claim **1**,

inserting said deodorizer device into a shoe, with the container in an inverted position such that the dispensing end of the container is lower than the opposite end;

stably resting the container in this inverted position on the inner surface of the shoe bottom; and

applying downward pressure to the opposite end of the container sufficient for discharging a quantity of deodorizer material into the shoe.

3. A device for deodorizing shoes, comprising:

a container containing a deodorizer material;

and a dispenser assembly at one dispensing end of the container for dispensing the deodorizer material therefrom;

said dispenser assembly including a feed tube having an inner region with one open end located within the container and an outer region with an opposite, dispensing end communicating with at least one discharge opening external of the container for discharging the deodorizer material laterally of the container;

said dispenser assembly further including an actuator attached at one end to said feed tube;

said actuator being in the form of a cap having a socket at a connecting end coaxial with, and dimensioned to receive said dispensing end of the feed tube and including a cylindrical wall formed with a slot for exposing the discharge opening with which the dispensing end of the feed tube communicates, the opposite, supporting end of said cap being dimensioned and configured to enable the container to stably rest in the inverted position within the shoe with said dispensing end of the container and said dispenser assembly disposed within the shoe, and the opposite end of the container extending outwardly of the shoe, to enable a quantity of the deodorizer material to be dispensed into the shoe by pressing against said opposite end of the container,

wherein the deodorizer material is an unpressurized liquid or powder and the dispenser assembly comprises a manual pump which is pumped by the actuator when the actuator is pressed against the inner surface of a shoe bottom.

4. The deodorizer device according to claim **3**, wherein the discharge opening is oriented to discharge the deodorizer material towards a shoe tip.

5. The deodorizer device of claim **3**, wherein the dispenser assembly comprises multiple discharge openings around the circumference of the dispenser assembly.

6. A method of deodorizing shoes, comprising:

providing a deodorizer device according to claim **3**,

inserting said deodorizer device into a shoe, with the container in an inverted position such that the dispensing end of the container is lower than the opposite end;

stably resting the container in this inverted position on the inner surface of the shoe bottom; and

applying downward pressure to the opposite end of the container sufficient for discharging a quantity of deodorizer material into the shoe.

7. The method of claim **6**, wherein the dispenser assembly includes a single discharge opening oriented to discharge the deodorizer material towards the tip of the shoe.

8. The method of claim **6**, wherein the dispenser assembly includes multiple discharge openings arranged around the circumference of the dispenser assembly to allow discharge of the deodorizer material in multiple directions within the shoe.

9. A device for deodorizing shoes, comprising:

a container containing a deodorizer material;

and a dispenser assembly at one dispensing end of the container for dispensing the deodorizer material therefrom;

said dispenser assembly including a feed tube having an inner region with one open end located within the container and an outer region with an opposite, dispensing end communicating with at least one discharge opening external of the container for discharging the deodorizer material laterally of the container;

said dispenser assembly further including an actuator attached at one end to said feed tube;

said actuator being in the form of a cap having a socket at a connecting end coaxial with, and dimensioned to receive said dispensing end of the feed tube and including a cylindrical wall formed with a slot for exposing the discharge opening with which the dispensing end of the feed tube communicates, the opposite, supporting end of said cap being dimensioned and configured to enable the container to stably rest in the inverted position within the shoe with said dispensing end of the container and said dispenser assembly disposed within the shoe, and the opposite end of the container extending outwardly of the shoe, to enable a quantity of the deodorizer material to be dispensed into the shoe by pressing against said opposite end of the container,

wherein the deodorizer material is a liquid and the container additionally contains a pressurized gas,

wherein the inner portion of said feed tube is integrally formed with a u-shaped extension such that the open end of the feed tube is near the dispensing end of the container, thereby enabling dispensation of substantially all of the deodorizer material when the container is inverted,

wherein the dispenser assembly comprises as internal 360° dispensing valve capable of dispensing the deodorizer material when the container is inverted and which remains in a closed position until the actuator is pressed against the inner surface of a shoe bottom, and

wherein the dispenser assembly comprises multiple discharge openings around the circumference of the dispenser assembly.

10. A method of deodorizing shoes, comprising:

providing a deodorizer device according to claim **9**;

inserting said deodorizer device into a shoe, with the container in an inverted position such that the dispensing end of the container is lower than the opposite end;

stably resting the container in this inverted position on the inner surface of the shoe bottom; and

applying downward pressure to the opposite end of the container sufficient for discharging a quantity of deodorizer material into the shoe.

11. The deodorizer device of one of claims **1**, **3**, or **9**, wherein the actuator is of a generally conical shape with the circumference of the supporting end of the actuator being greater than the circumference of the connecting end.

12. The deodorizer device according to one of claims **1**, **3**, or **9**, wherein the cap is in the configuration of a shoe.

13. The deodorizer device according to one of claims **1**, **3**, or **9**, wherein the end of the container opposite the dispensing end is of a done configuration.

14. The deodorizer device according to one of claims **1**, **3**, or **9**, wherein the deodorizer material is a liquid comprising 70% ethanol, 23% water, 3.2% preventol R80,2% NP10, 1% orange terpenes, 0.5% pine oil, 0.15% perfume, 0.1% citric acid, and 0.05% EDTA.

15. The method of one of claims **2**, **6**, or **10**, wherein the actuator cap has the configuration of a shoe.

16. The method of one of claims **2**, **6**, or **10**, wherein the end of the container opposite the dispensing end is of a dome configuration.

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Rosenberg

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(54) **COMPOSITION FOR DESORBING BACTERIA**

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(58) **Field of Search** 514/642, 358, 514/635; 424/52, 54

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(57) **ABSTRACT**

The invention provides a composition for desorbing bacteria from solid surfaces and from living tissues, which is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime, the composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase, comprising a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydrocarbon or a mixture thereof; and

c) about 0.003 to about 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of the temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

13 Claims, No Drawings

COMPOSITION FOR DESORBING BACTERIA

This application is a continuation, of application Ser. No. 07/330,262 filed Mar. 29, 1989, now abandoned.

The present invention relates to novel compositions which desorb bacteria and other microorganisms from solid surfaces and from living tissue.

The main use contemplated for the compositions of the present invention is in oral hygiene. Compositions of the invention can also be used in entirely different applications, where microorganisms are to be removed from surfaces to which they are attached, or where enhanced microbial adhesion to oil droplets is desired.

According to the present invention there is now provided a composition for desorbing bacteria from solid surfaces and from living tissues, which is in the form of a two-phase preparation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime said composition comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase, comprising a vegetable oil, a mineral oil, a pharmacologically acceptable aliphatic hydrocarbon or a mixture thereof; and

c) about 0.003 to about 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

Amongst edible oils, which can be used to form said water immiscible oily phase there may be mentioned oils such as olive oil, corn oil, coconut oil, soybean oil, safflower oil. There can also be used a wide variety of pharmacologically acceptable hydrocabons such as octane, decane, tetradecane, hexadecane, xylene, white mineral oil, and mixtures thereof etc.

Amongst suitable colors, there may be mentioned colors of triphenylmethane, naphthol, xanthene, monoazo, pyrazol, anthraquinone and cationic colors; examples include Food Blue 2 and its ammonium salt; D&C Yellow No. 7, D&C Yellow No. 10, D&C Yellow Nos. 4, 6, 22, 28, 33 or 40; D&C Green No. 5, D&C Orange No. 11, D&C Red Nos. 19 and 37, Basic Blue Nos. 6, 9, 41, 99, etc.

Preferably said amphipathic cationic moiety is selected from pyridinium core surface-active cationic molecules such as cetylpyridinium chloride, laurylpyridinium chloride, etc.; from chlorhexidines such as chlorhexidine, its diacetate, chlorhexidine digluconate, chlorhexidine dihydrochloride; from monalkyl quaternary ammonium compounds (quats) such as benzalkonium chloride, cetalkonium chloride and bromide, lauralkonium chloride and bromide, soytrimonium chloride, PEG-5 stearyl ammonium lactate; from dialkyl quaternary ammonium compounds (diquats) such as dilauryl dimonium chloride, dicetyl dimonium chloride and bromide, dequalinium chloride, soyamido propyl benzyldimonium chloride, quaterniums such as quaternium 15 and polyquaterniums, etc; amine fluorides, from cationic polysaccharides, such as chitosan and its derivatives; from cationic polypeptides, such as poly L-lysine, poly D-lysine, lysozyme.

In especially preferred embodiments of the present invention said amphipathic cationic moiety is selected from cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme.

The invention also provides a method for enhancing microbial adhesion at an oil-water interface comprising admixing an aqueous microbial suspension and oil in the presence of an amphipathic cationic moiety.

In U.S. Pat. No. 4,525,342, Weiss et al. have described an essentially detergent free mouthwash based on a combination of an oily phase and an aqueous one. This composition must be marketed in a two-compartment squirt bottle, and the user swishes the two phases in his mouth in order to attain the desired effect. The composition is based on the attachment of amphipathic substances to the oil droplets during the swishing action, thus coating the oil droplets. The presence of detergents is reported to be deleterious in said patent. Various deleterious physiological effects have been reported regarding the use of detergents in the oral cavity. It has been reported that the presence of detergents prevents, or at least decreases adhesion of microorganisms to oil droplets as is taught, e.g. by the statement: "The aqueous bacterial suspension must be free of surfactants, which inhibit the assay" (M. Rosenberg, 1984. Bacterial Adherence to Hydrocarbons: A Useful Technique for Studying Cell Surface Hydrophobicity. FEMS Microbiology Letters 22, 289–295). In a recent review on hydrophobic interactions and adhesion, the authors write: "Our appreciation of the extent to which hydrophobic interactions mediate various bacterial adhesion phenomena could be greatly augmented by including agents that interfere with hydrophobic interactions (e.g., surfactants, chaotrophic agents) in adhesion assays (M. Rosenberg and S. Kjellerberg, 1986, Hydrophobic Interactions: Role in Bacterial Adhesion. Advances in Microbial Ecology 9, 353–393). Moreover, in a previous study which did examine the effect of some cationic amphipathic antibacterial agents on adhesion of relatively non-hydrophobic *Escherichia coli* strains to xylene, the cells were exposed to the cationic agents, but the cationic agents were subsequently removed by washing the cell pellet prior to addition of the xylene, presumably to avoid inhibition of the adhesion in the presence of the cationic agents (B. M. A. El-Falana, D. T. Rogers, A. D. Russell and J. R. Furr. 1985 Effect of Some Antibacterial Agents on the Hydrophobicity of Wild-Type and Envelope Mutant of *Escherichia coli*. Current Microbiology 12, 187–190). Similarly, chlorhexidine, an amphipathic, surface-active cationic agent commonly incorporated into mouthwashes has been shown in many studies to inhibit microbial adhesion to surfaces. For example, J. McCourtie, T. W. MacFarlane and L. P. Samaranayake (Effect of saliva and serum on the adherence of Candida species to chlorhexidine-treated denture acrylic, J. Med. Microbiology, 21:209–213, 1986) showed that treatment of saliva- or serum-coated acrylic with chlorhexidine gluconate reduced adherence by between 19 and 86%.

Moreover, whereas salts such as sodium chloride have been generally considered to enhance microbial adhesion to surfaces, it has now been found that the presence of inorganic cations, such. as sodium and magnesium cations, inhibit the stimulation of adhesion brought about by cationic moieties such as cetylpyridinium, chlorhexidine, chitosan, poly L-lysine, poly D-lysine and lysozyme.

The main use of the compositions is in the form of mouthwashes, which effectively remove a large percentage of microorganisms, debris and other odor-causing materials from the surface of teeth and from the oral cavity. Thus especially preferred embodiments of the present invention relate to a mouthwash as defined above comprising about 0.003 to about 0.5 w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

The novel compositions comprise, in combination, an organic water-immiscible phase and an aqueous phase, and a small quantity of an amphipatic cationic moiety, which is adequate to form upon vigorous mixing an emulsion of brief life-time. In addition, the composition may comprise additional cationic agent or agents which enhance adhesion to oil droplets, as shall be illustrated later. Optimally, the composition should be relatively free from interfering cations, such as sodium, potassium and magnesium and therefore preferably contain only up to 0.3% of inorganic salts.

In use, the two phases are mixed by shaking, and an emulsion is formed which is used for swishing in the mouth, and which has a limited life time, of the duration of about 10 seconds to about 30 minutes. A small amount of amine fluoride which tends to stabilize the emulsion for a brief period of time and has other beneficial (e.g. anticaries, antibacterial) effects may also be added.

There may also be incorporated certain additives such as fragrances, colors and the like.

While the invention will now be described in connection with certain preferred embodiments in the following examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

The following experimental results illustrate the invention, both in regard to the specific formulations and also as regards the mechanism of action of the emulsions of the present invention as regards removal of dental plaque and adhering bacterial and other microorganisms.

The mouthwash compositions of the invention preferably comprise a small quantity of an amphipatic cationic moiety which is preferrably a surface-active agent which is able to form an emulsion of limited life time. The emulsion ought to be formed upon shaking of the container which contains the two separate phases, and it should preferably remain in emulsion form for at least about 20 seconds. Experiments have shown that compositions of the invention form temporary emulsions which separate after a period of about 10 seconds to 10 minutes.

We have found the unexpected result that small amounts of cationic surface-active agents promote, rather than inhibit, adhesion of bacteria and oral debris, to oil droplets. We have also found the surprising and unexpected results that sodium and magnesium chloride inhibit, rather than enhance, microbial adhesion in the presence of such cationic agents as (cetylpyridinium chloride, chlorhexidine digluconate and chitosan.

The following experiments demonstrate the ability of the cationic surfactant, cetylpyridinium chloride, to enhance microbial adhesion to oil droplets in various experiments. In one experiment *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30 C in brain heart infusion broth. The cells were harvested by centrifugation and washed twice in 0.2% saline. The cells were then suspended in 0.2% saline to a corrected optical density of about 15 at 400 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% detergent in water (or water for control) and 0.24 ml water. Following brief mixing, 0.2 ml of oil was added (vs. controls with no oil added) and the mixtures vortexed vigorously for 2 min. Following about thirty minutes, the corrected turbidity of the lower aqueous phase was measured at 400 nm. Adhesion is calculated as the percent drop in turbidity following the mixing procedure. The results are summarized in the following Table.

TABLE 1

Adhesion RAG-1 to various oils in the presence of cetylpyridinium chloride

	Percent adhesion to:		
Additions	n-nexadecane	mineral oil 50	soybean oil
Cetylpyridinium chloride	83	80	47
None	27	5	32
Sodium dodecyl sulphate	0	0	0
Tween 20	0	0	0

As can be seen from the above Table, in each case, cetylpyridinium chloride greatly enhanced bacterial adhesion to the oil droplets. This phenomenon was also observed microscopically. Enhanced adhesion was not observed for non-cationic surface-active agents: adhesion to all three test oils in the presence of the anionic sodium lauryl sulphate or the nonionic Tween 20, added at the same concentration as the cetylpyridinium chloride (w/v), was 0%. Morever, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of *Escherichia coli* CSH 57 to hexadecane could be increased from 0 to 98% when 0.08% cetylpyridinium chloride was present; adhesion of the yeast *Candida alpicans* rose from 20 to 100% in the presence of 0.16% chlorhexidine digluconate. Adhesion of *Acinetobacter calcoaceticus* MR-481 rose from 0 to 97% in the presence of 0.18% of cetylpyridinium chloride.

In another experiment, a volunteer swished his mouth with 0.2% saline solution (10 ml) for thirty seconds. To 1 ml of the expectorate were ad ed 0.18 ml of 0.5% cetylpyridinium chloride (water in the case of the control) and 0.44 mm 0.2% saline. Mineral oil 50 (0.2 ml) was then added and the mixtures vortexed for 2 minutes. Following phase separation, the mixtures were examined. Whereas the turbidity of the expectorate was greatly reduced in the presence of cetylpyridinium chloride, presumably due to adsorption of bacteria and debris to the oil droplets, in the absence of this detergent, no increase in turbidity was observed.

The ability of another cationic surface-active agent, chlorhexidine digluconate, to enhance microbial adhesions to oil droplets is shown in the following experiment. *Actnetobacter calcoaceticus* RAG-1 (ATCC 31012) cells were grown overnight with vigorous shaking at 30 C in brain heart infusion broth. The cells were harvested, washed by centrifugation, and suspended in 0.2% saline to a corrected optidal density of ca. 18 at 0 nm (1 cm light path). To 1.2 ml of suspended bacteria in 4 ml square disposable polystyrene cuvettes were added the following: 0.16 ml of 0.5% chlorhexidine digluconate in water (or water for control) and 0.24 ml water. To each cuvette 0.2 ml of oil was added (vs. control with no oil added) and the mixtures vortexed vigorously for 2 min. After about thirty minutes, the turbidity of

the lower aqueous phase was measured at 400 nm and corrected for non-linearity by a standard curve. Adhesion is calculated as the percent drop in corrected turbidity following the mixing procedure. The results are summarized in the following Table.

TABLE 2

Adhesions of RAG-1 to various oils in the presence of chlorhexidine digluconate

	Percent adhesion to			
Additions:	n-hexadecane	isohexadecane	Sigma* soybean oil	Commerical soybean oil
Chlorhexidine digluconate	95	94	90	89
None	50	66	54	49

*St. Louis, Mo.

As can be seen from the above Table, in each case chlorhexidine digluconate greatly enhances bacterial adhesion to the oil droplets. Moreover, the enhanced adhesion can be shown also for microbial cells other than RAG-1 which have little or no affinity for oil droplets. For example, the adhesion of *Escherichia coli* CSH 57 to hexadecane could be increased from 0 to 98% when 0.1% chlorhexidine digluconate was present.

Adhesion to oil droplets may also be enhanced by polymeric cationic agents, such as chitosan. For example, the adhesion of *Escherichia coli* CSH 57 to hexadecane could be increased from 0 to 97% when 0.015% chitosan was present; adhesion of the yeast *Candida albicans* rose from 20 to 95% in the presence of 0.025% chitosan. Adhesion of *Acinetobacter calcoaceticus* MR-481 rose from 0 to 99% in the presence of 0.019% of chitosan.

The compositions of the invention are suitable for a variety of applications. As a two-phase mouthwash, containing an aqueous phase and an oil phase, the presence of adhesion-enhancing cationic agent (or combination thereof) could remove and bind high levels of oral microorganisms and debris.

The enhanced Adhesion of RAG-1 cells to oils in the presence of cationic surfactants similarly teaches the use of cationic surface-active agents to enhance microbial adhesion to oils for commercial purposes, such as immobilization of cells onto oil droplets, or of cells, cell walls, and the like for enhanced adhesion to adjuvant oils in the vaccination field.

In general, salts such as sodium chloride, magnesium chloride and various phosphates, enhance adhesion of microorganisms to surface by countering the net negative charges which generally exist on cell surfaces, and often on substrata surfaces. Weiss et al. include in their U.S. Pat. No. 4,525,342, a requirement for salt. However, as stated, it has now been found that the enhancement of microbial adhesion to oils observed in the presence of cetylpyridinium chloride, chlorhexidine gluconate and chitosan is inhibited by sodium chloride and magnesium chloride.

For example, adhesion of *C. albicans* to hexadecane in the presence of 0.16% cetyl pyridinium chloride fell from 100% to 18% when 0.8% NaCl was present.

In order to obtain a picture as to the efficacy of the compositions of the invention for oral use, these were compared with existing mouthwashes as regards their ability to remove a given bacterial film from a solid surface.

There were used a number of commercial products and these were compared with the preparation of the invention defined below as ODEX-35. The following describes and illustrates in detail specific features of the invention:

EXAMPLE ODEX-35

Aqueous phase:

sodium chloride	0.2% w/w
sodium saccharin	0.05% w/w
FD&C Blue No. 1	0.0001% w/w
cetyl pyridinium chloride	0.03% w/w
water	to 100%

Oil phase

soybean oil	35.0% w/w
essential oil (mint)	1.5% w/w

This type of formulation has several advantages. First, the presence of cetylpyridinium chloride results in adsorption of much higher levels of bacterial and oral debris, than when it is absent. Second, the mixture can be presented in a single, regular container, e.g. bottle, obviating the need for a double compartment, double squirt bottle. The user simply shakes for several seconds, the amount of cetylpyridinium chloride is sufficient to provide an emulsion with limited stability which enables the user to pour the desired quantity of mixture and use it directly to swish and gargle. An added advantage of the formulation is that the cetylpyridinium chloride enhances the dye binding capacity of the bacteria and debris so that they are clearly colored blue by the dye, facilitating their observation. Moreover, the dye and cetylpyridinium chloride may act together in a synergistic fashion as antibacterial agents.

In one experiment, the formulation was mixed, and 10 ml immediately poured into a small cup. Volunteers swished and gargled with the mixture for a thirty second period. Following swishing, the mixture was expectorated into test tubes. It was clearly observed in all cases that the oral bacteria and debris, dyed blue, rose together with the yellow oil droplets, creating a green upper oil:in:water emulsion which was stable for hours. Bacteria, and bacteria-coated epithelial cells were microscopically observed adhering to the oil droplets. The aqueous phase lost its blue color, presumably due to the binding of the dye to the great quantities of bacteria and debris removed onto the oil droplets during the mixing procedure. A drop in the level of bad breath of the volunteers was evident for hours following the swishing procedure. Moreover, volunteers reported a long lasting fresh feeling in their mouths, and did not report any oily taste, despite the high level of oil in the preparation. These observations could not be reproduced if either the oil phase, or the cationic surface-active agent were absent.

It should be emphasized that the compositions of the invention also have a wide range of uses in enhanced desorption of microorganisms for host, industrial and environmental surfaces onto oil droplets. To illustrate this point, we have succeeded in demonstrating that the above formulation is highly effective in removing adsorbed bacteria from a model surface, i.e. polystyrene, as described in the following experiment.

One ml of a very turbid suspension of RAG-1 cells in 150 mM phosphate buffer was added to a polystyrene cuvette (4 ml capacity). Following 1 minute vortexing, the RAG-1

cells were poured off, leaving a film of adherent cells. The cuvettes were washed briefly with tap water. To each cuvette, 1 ml of mouthwash (when indicated, diluted according to instructions) or water (control) were added and the cuvettes vortexed for 2 minutes. The mouthwash was poured off, 1 ml fresh mouthwash was added, and the cuvettes again vortexed for 2 minutes. The mouthwash was poured of, and the remaining bacterial film stained by adding Gentian Violet to the cuvettes, followed by washing to remove excess stain. The amount of bacterial film was ascertained by measuring the optical absorbance (585 nm) of the dried cuvettes from both sides in a Uvikon spectophotometer. The results are presented in Table 3.

From the following Table it can be seen that among the commercial mouthwashes tested, several (Eludril, P. F. Medicament, 125, rue de la Faisanderie, Paris, France; Tayadent, Taya Ltd., Petach Tiqua, Israel and Act®, Johnson & Johnson, New Brunswick, N.J. 08903) were less able than water to desorb the bacterial film. Menthol Chloraseptic® (Norwich Eaton Pharmaceuticals, Inc., Norwich, N.Y. 13815) and Veadent® (Glaxo, under licence from Vipont Laboratories Inc., Ft. Collins, U.S.A.)desorbed less than 10% of the adherent bacteria. Cepadont removed 29% of the film. Only ODEX-35 removed the large majority (87%) of the bacterial film. Moreover, ODEX-35 without the oil phase was relatively ineffective (26%) as compared to both phases together (87%).

TABLE 3

Mouthwash	$OD_{585\ nm}$ (mean)	Percent removal relative to water control
Control (water	0.300	—
Eludril	0.625	—
Tayadent	0.376	—
Act (green)	0.344	—
Menthol Chloraseptic	0.288	4
Veadent	0.281	6
Cepadont	0.213	29
ODEX-35, aqueous phase only	0.223	26
ODEX-35	0.039	87

In order to further test compositions of the kind described above for use as mouthwashes, several in vivo tests were undertaken. In one of these, various compositions were prepared and swished for two thirty second periods by a volunteer at bedtime. The following morning, the volunteer swished with ten ml of sterile milk, and 3 ml of the expectorate was mixed in a test tube with 0.12 ml of 0.1% methylene blue. The test tube was allowed to stand at room temperature and the time required for the bottom of the test tube to turn white was observed. It has been previously shown that the time required for a color change from blue to white at the bottom of the test tube correlates highly with microbial counts, thus a table was used to convert the time for color change into relative microbial counts. The results are presented in Table 4, together with the time required for the color change, and the percentage of microorganisms remaining, as compared to a no-mouthwash control. Several of the compositions could not be mixed or were unattractive (samples 3 and 14) and were not tested in the mouth. Others (samples 16 and 23A) were tried several times.

TABLE 4

	Ingredients (% w/w)											
Sample Number	Soybean oil	Mineral oil	Peppermint oil	Cetyl pyridinium chloride	Sodium lauryl sulphate	Chlorhexidine digluconate	Sodium saccharin	Xylitol	Aspartame	Sodium chloride	Ethanol (absolute)	Tween 20
1	35	—	1.5	0.05	—	—	0.05	—	—	—	—	—
2	35	—	1.5	0.025	—	—	0.05	—	—	—	—	—
3	35	—	1.5	—	—	0.04	0.05	—	—	—	—	—
4	35	—	1.5	0.025	—	0.04	0.05	—	—	—	—	—
5	35	—	1.5	—	—	0.2	—	5	—	—	—	—
6	35	—	1.5	—	—	0.2	—	—	0.1	—	—	—
6	35	—	1.5	—	—	0.2	—	—	0.1	—	—	—
12	35	—	1.5	—	—	0.1	—	—	0.1	—	—	—
13	35	—	1.5	—	—	0.05	—	—	0.1	—	—	—
14	35	—	1.5	—	—	0.025	—	—	0.1	—	—	—
15	35	—	1.5	0.05	—	0.1	—	—	0.1	—	—	—
16	35	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
16	35	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
22A	25	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
23A	15	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
23A	15	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
24A	5	—	1.5	0.05	—	0.05	—	—	0.1	—	—	—
25	—	—	0.1	—	—	0.1	—	—	0.1	—	—	0.4
26	—	—	0.1	0.05	—	0.05	—	—	0.1	—	—	0.4
31	—	15.0	1.5	0.05	—	0.05	—	—	0.1	—	—	—
32	5	—	0.75	0.05	—	0.05	—	—	0.1	—	—	—
34	33	—	1.5	—	0.10	—	—	—	0.1	—	—	—
35	35	—	1.5	0.05	—	—	0.05	—	—	0.2	—	—
36	35	—	1.5	0.05	—	—	0.05	—	—	—	16.0	—

TABLE 4-continued

Sample Number	Ingredients (% w/w) FD&C Blue No. 1 (Food Blue 2: CI 42090)		Water	Results: Time for color change	% residual micro-organism
1	0.00002	to	100%	30'	36.0
2	0.00002	to	100%	14'	61.0
3	0.00002	to	100%	—	—
4	0.00002	to	100%	10'	77.0
5	0.00002	to	100%	>80'	<18.0
6	0.00002	to	100%	90	17.0
6	0.00002	to	100%	420'	6.0
12	0.00002	to	100%	>100'	<15.0
13	0.00002	to	100%	35'	32.0
14	0.00002	to	100%	—	—
15	0.00002	to	100%	>124'	13.0
16	0.00002	to	100%	>160'	11.0
16	0.00002	to	100%	>120'	<14.0
22A	0.00002	to	100%	>142'	<12.0
23A	0.00002	to	100%	>264'	<8.0
23A	0.00002	to	100%	>226'	<9.0
24A	0.00002	to	100%	129'	13.0
25	0.00002	to	100%	<30'	>36.0
26	0.00002	to	100%	32'	34.0
31	—	to	100%	90'	17.0
32	0.00002	to	100%	119'	<14.0
34	0.00002	to	100%	31'	36.0
35	0.00002	to	100%	35'	32.0
36	0.00002	to	100%	19'	49.0

It is clear from Table 4 that a positive interaction exists between cetylpyridinium chloride and chlorhexidine. Thus, whereas sample 1 containing only cetylpyridinium chloride (0.05%) as cationic agent and sample 13 containing only chlorhexidine digluconate (0.05%) as cationic agent left a high percentage of residual microorganisms in the mouth (over 30%), a combination of 0.05% cetylpyridinium chloride and 0.05% chlorhexidine (sample 16) was highly effective, leaving less than 14% residual microorganisms in one instance, and 11% in another. Moreover, the cetylpyridinium chloride has a better emulsifying ability, whereas chlorhexidine digluconate has better antibacterial activity. Thus, the combination of both together with the oil is highly effective in obtaining a composition which can be effectively emulsified by shaking for a brief period, combined with potent antibacterial ability. It should be noted that compositions containing from 5–35% (w/w) of soybean oil, cetylpyridinium chloride, and chlorhexidine were highly effective (samples 15,16, 22A–24A). Another advantage of the two-phase oil:water combination is that moieties which generally require emulsification in regular mouthwashes can be added to the oil phase. For example, in order to introduce peppermint oil into the aqueous phase, it must be solubilized e.g. with Tween. This causes a great reduction in activity of the formula, by inactivating the cations (e.g. chlorhexidine) present (see for example the poor performance of samples 25 and 26, even though antibacterial cations are present).

Thus, when antibacterial cations such as chorhexidine are present in the aqueous phase, it is preferable to add the oil-soluble components to the oil phase. Such components include flavors, antioxidants (e.g. Vitamin E, BHA, BHTA), and essential oils which have antibacterial activity, e.g. eucalyptis oil, cinnamon oil.

On the other hand, samples in which the essential oil (peppermint) was solubilized in the aqueous phase using Tween 20 (samples 25 and 26) were not effective, even though either 0.1% chlorhexidine (sample 25) or a combination of 0.05% of both cetylpyridinium chloride and chlorhexidine (sample 26) were present.

Bad breath is known to be chiefly due to microbial activity in the oral cavity. Sample 23A was tested against a leading commercial mouthwash (Listermint, Warner-Lambert Health Care Ltd., Eastleigh, Hants, England) for its ability to reduce bad breath levels, as determined by oral sulphide levels, using a sulphide monitor, (Interscan Model 1170, Interscan Ltd., Chatsworth, Calif.). One-quarter inch plastic straws are inserted into the sample inlet and the volunteer allows the end of the straw to enter the oral cavity, and breathes through his nose. The peak and steady-state value are recorded in parts per billion sulphide equivalents. Volunteers swished with either mouthwash for two thirty second periods at bedtime and tested peak and steady-state sulphide levels immediately upon awakening. Sample 23A reduced peak levels by 74%, and steady-state levels by 70%, as compared to no-mouthwash controls; in contrast, Listermint reduced peak levels by 51% and steady state levels by 45% as compared to no-mouthwash controls. Moreover, in a comparison of the ability of sample 23A to remove bound microorganisms from a solid surface, in an experiment analogous to that described in Table 3, sample 23A was superior to a wide variety of commercial mouthwashes in its ability to desorb bound cells.

As an additional example, we have tested formulations whose oil phase contains a combination of olive oil, peppermint oil and eucalyptus oil. One formulation, designated ODEX, has the following composition:

Aqueous Phase:

cetylpyridinium chloride	0.05% (w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.75% (w/w)

Oil Phase:

olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

Another formulation, designated ODEX+ has the following composition:
Aqueous Phase:

cetylpyridinium chloride	0.05% (w/w)
chlorhexidine digluconate	0.05% (w/w)
aspartame	0.1% (w/w)
FD&C blue no. 1	0.1% of a 1% w/w aqueous solution
water	84.70% (w/w)

Oil Phase:

olive oil	13.50% (w/w)
peppermint oil	1.00% (w/w)
eucalyptus oil	0.5% (w/w)

The ability of these formulations to desorb microorganisms from polystyrene was tested as described in reference to Table 3. ODEX and ODEX+ removed 98% and 92%, respectively, of the bound RAG-1 bacteria.

Moreover, ODEX was tested in its ability to reduce bad breath, as compared to the following controls: (i) a commercial mouthrinse, Cepadont; (ii) a control in which the oil phase was entirely omitted; (iii) a control in which the olive and peppermint oil were omitted, and the eucalyptus oil solubilized in 6% Tween 20; and (iv) no mouthrinsing control. Peak morning odor was measured using the Interscan 1170 sulphide analyzer. Two volunteers rinsed, prior to bedtime, for two consecutive 30 second periods. Immediately upon awakening, peak sulphide was measured. The results are summed up in Table 5 below:

TABLE 5

Overnight Effect of ODEX on Bad Breath

	Parts per billion sulphide equivalents	
FORMULATION TESTED	Volunteer 1	Volunteer 2
ODEX	25	20
(i) Cepadont	140	70
(ii) No oil phase	70	40
(iii) Solubilized eucalyptus oil	60	—
(iv) No mouthrinsing	117	150

These data show that the combination of aqueous phase and non-emulsified oil phase containing eucalyptus oil was more effective than any of the controls.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative examples and that the present invention may be embodied in other specific forms without departing from the essential attributes thereof, and it is therefore desired that the present embodiments and examples be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

What is claimed is:

1. A composition for desorbing bacteria from solid surfaces and from living tissues, comprising:

a) about 50 to about 97% w/w of an aqueous phase;

b) about 3 to about 50% w/w of a water immiscible oily phase; and

c) about 0.003 to about 2.0% w/w of an amphipathic cationic moiety in an effective amount to enable the formation of a temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof,

wherein said composition is in the form of a two-phase preparation not requiring physical separation, and which upon shaking forms a temporary oil-in-water emulsion of limited lifetime.

2. A composition for desorbing bacteria from solid surfaces and from living tissues, according to claim **1** wherein said amphipathic cationic moiety is selected from the group consisting of pyridinium core surface-active cationic molecules, chlorhexidines, monalkyl quaternary ammonium compounds, dialkyl quaternary ammonium compounds, quaterniums and polyquaterniums, amine fluorides, cationic polysaccharides, and cationic polypeptides.

3. A composition for desorbing bacteria from solid surfaces and from living tissues, according to claim **1** wherein said amphipathic cationic moiety is selected from the group consisting of cetylpyridinium chloride, a chlorhexidine compound, chitosan, chitin derivatives, poly L-lysine and lysozyme.

4. A composition according to claim **1**, wherein the oily phase is selected from the group consisting of olive oil, corn oil, coconut oil, soybean oil, safflower oil, octane, decane, tetradecane, hexadecane, white mineral oil and a mixture of two or more thereof.

5. A composition according to claim **1** wherein said amphipathic cationic moiety is a chlorhexidine compound.

6. A composition according to claim **1** wherein said amphipathic cationic moiety is chitosan.

7. A mouthwash according to claim **1** comprising about 0.003 to about 0.5 w/w of an amphipathic cationic moiety in an effective amount to enable the formation of said temporary oil-in-water emulsion which emulsion breaks down and separates within a period of about 10 seconds to thirty minutes of the formation thereof.

8. A composition according to claim **1** containing up to 0.3% of inorganic salts, selected from the group consisting of phosphate, sodium or magnesium chloride and fluorides.

9. A composition according to claim **1** which contains about 0.005 to 0.5% cetylpyridinium chloride.

10. A composition according to claim **1** which contains about 0.005 to 0.5% cetylpyridinium chloride and about 0.005 to 0.5% of a chlorhexidine compound.

11. A composition according to claim **1** further comprising a pharmaceutically acceptable dye which binds to microorganisms and debris.

12. A method of removing microorganisms from a surface comprising the steps of:

a) shaking a two-phase mixture including an aqueous phase, a water-immiscible oil phase, and an amphipathic cationic moiety in sufficient amount to form, upon shaking, a temporary oil-in-water emulsion which breaks down and separates within a period of from about ten seconds to thirty minutes; and

b) applying said temporary oil-in-water emulsion to said surface.

13. The composition according to claim **1**, wherein said water immiscible oily phase is selected from the group consisting of a vegetable oil, a mineral oil, a pharmaceutically acceptable aliphatic hydrocarbon, and a mixture thereof.

* * * * *

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(54) Abstract Title: **Bottle with a flow impeding device for dispensing of two phase liquids**

(57) A bottle 100 for containing and dispensing of a non-uniform liquid comprising a lower-phase 20 and an upper-phase 10 where the lower-phase 20 is a liquid of a higher specific-weight than the upper phase 10. The bottle 100 may comprise a flow-impeding device 150, where the flow-impeding device 150 impedes the flow of liquid that is dispensed from the bottle 100 to allow impeding the dispensing of the uppermost layer, which may be the upper-phase layer 10 from said bottle 100. The flow impeding device 150 takes the form of reduced aperture 115 (see figs 6, 7) having at least one protruding sealing member 152 which creates at least one cavity 30. The flow impeding device may be in the form of a separate device (see ref 50, figs 11, 12) inserted into the bottle 100 which is made from elastic materials enabling it to change dimensions according to pressure applied thereon. The bottle 100 may also have an indented portion (see 9, 10) which enhances flow of the lower phase 20 towards the opening of the bottle by leaving less space for the lower phase 20 to remain in once the liquid is poured. The bottle 100 is preferably put to storing and dispensing two phase mouthwash.

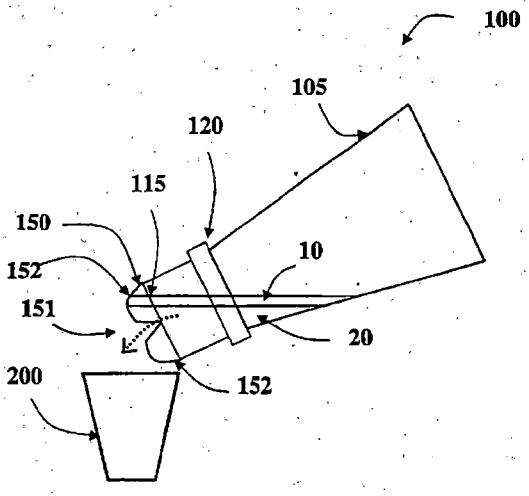

Fig. 5

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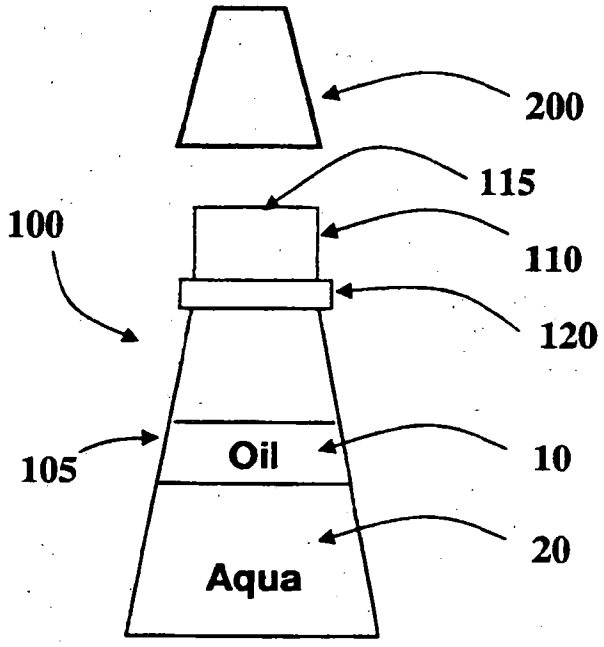

Fig. 1

(Prior Art)

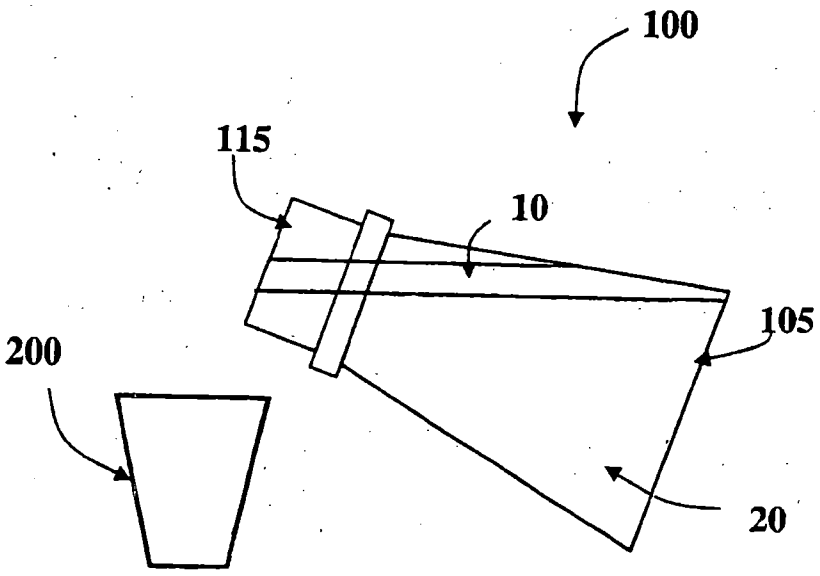

Fig. 2
(Prior Art)

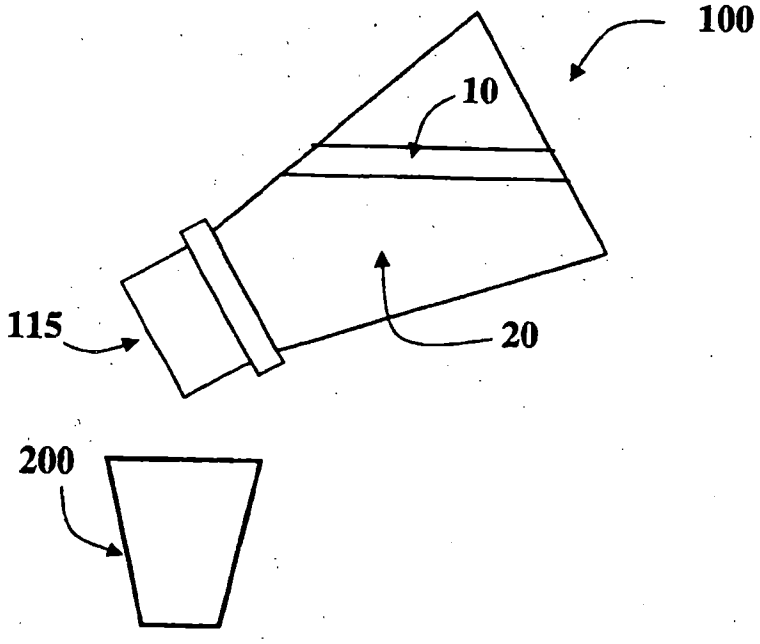

Fig. 3
(Prior Art)

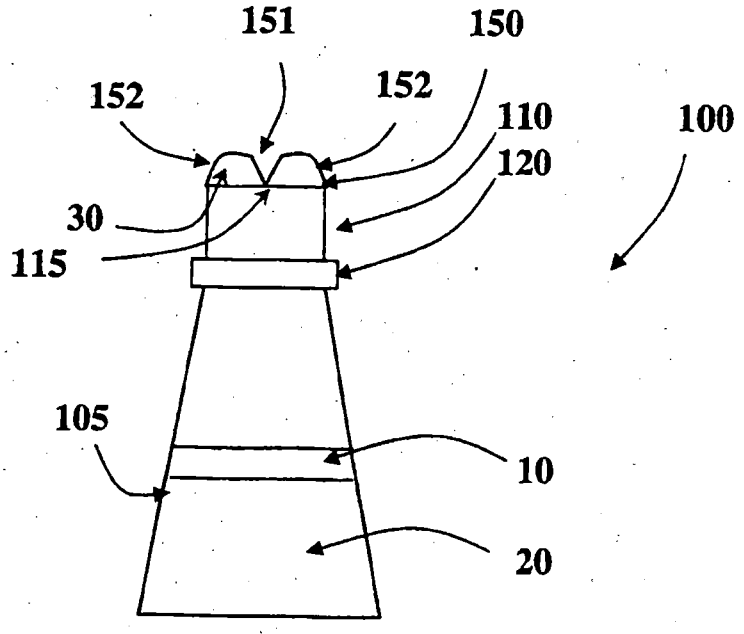
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Fig. 4

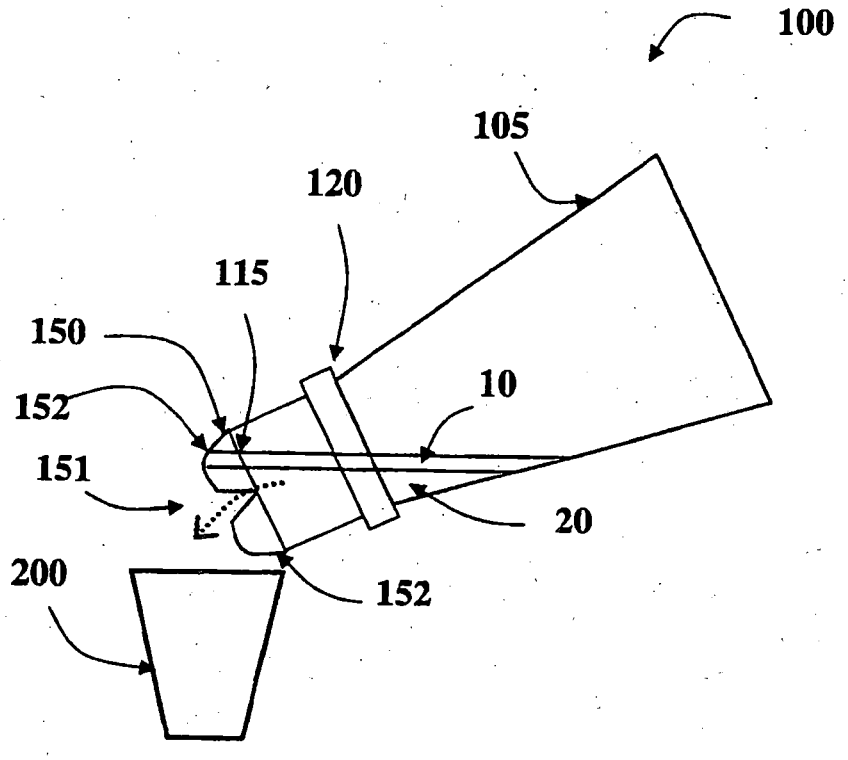
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Fig. 5

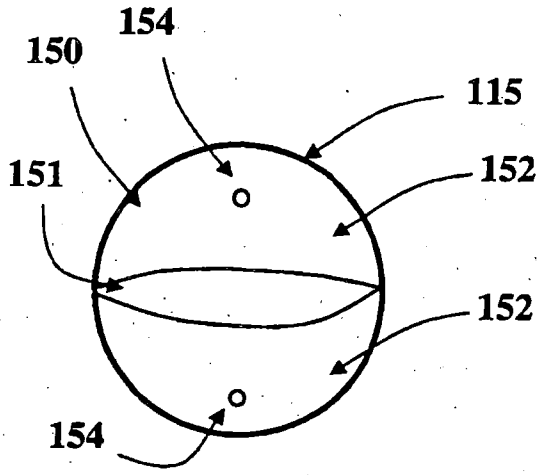

Fig. 6

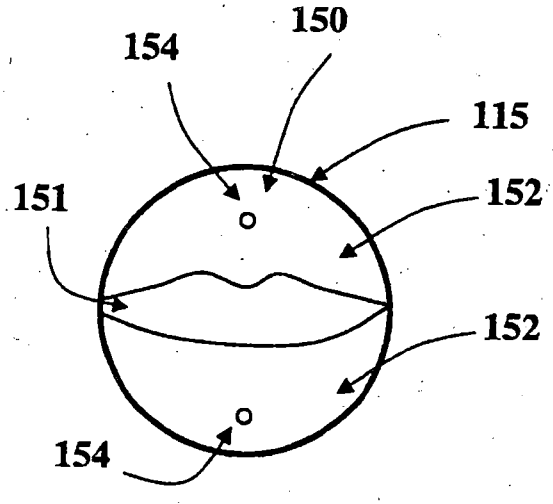

Fig. 7

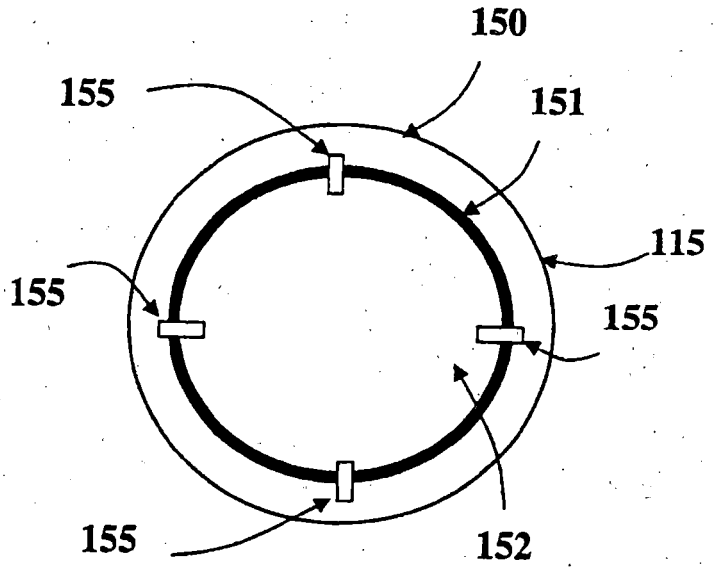

Fig. 8A

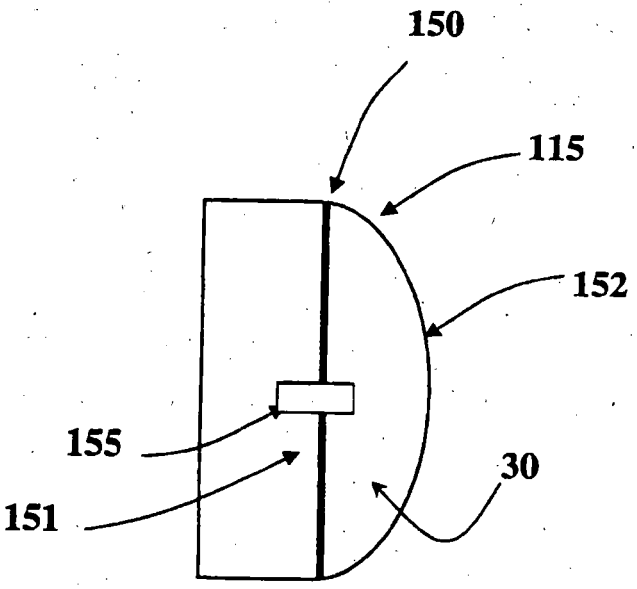

Fig. 8B

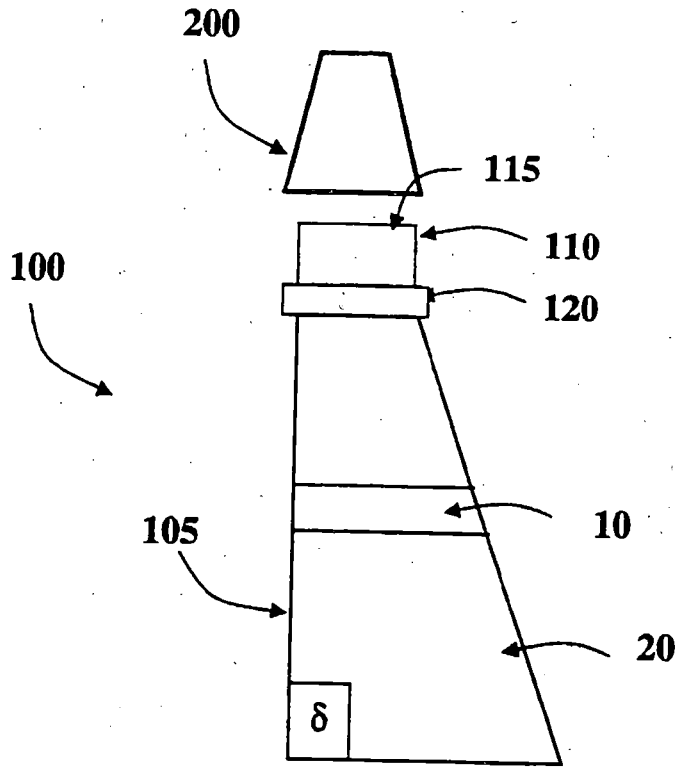

Fig. 9

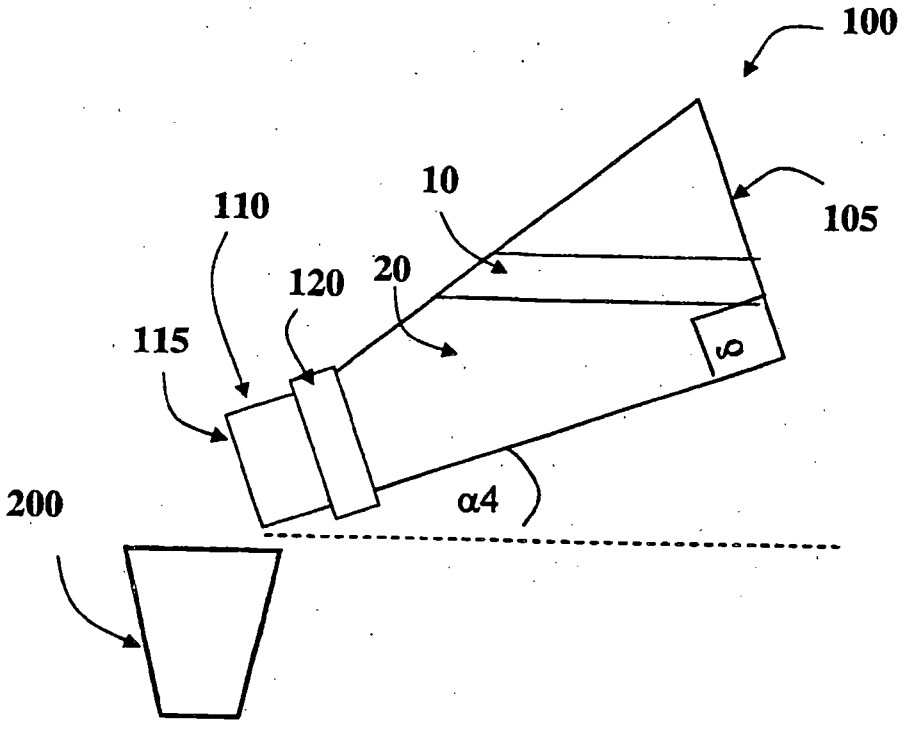

Fig. 10

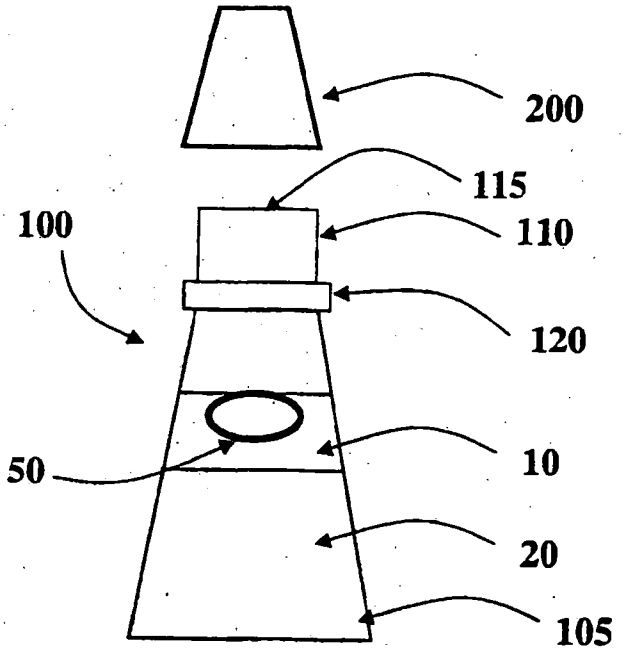

Fig. 11

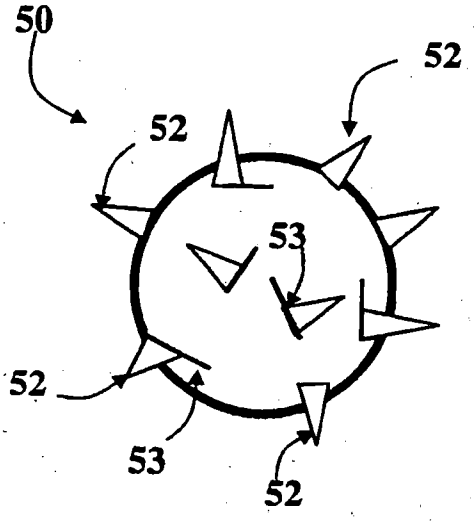

Fig. 12A

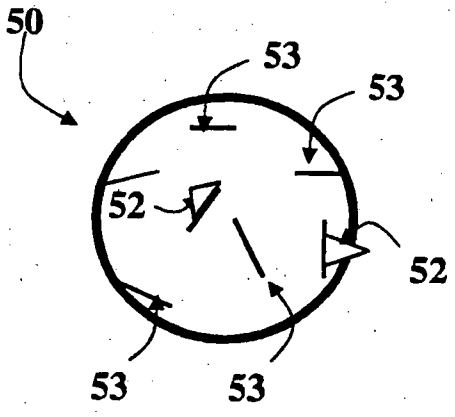

Fig. 12B

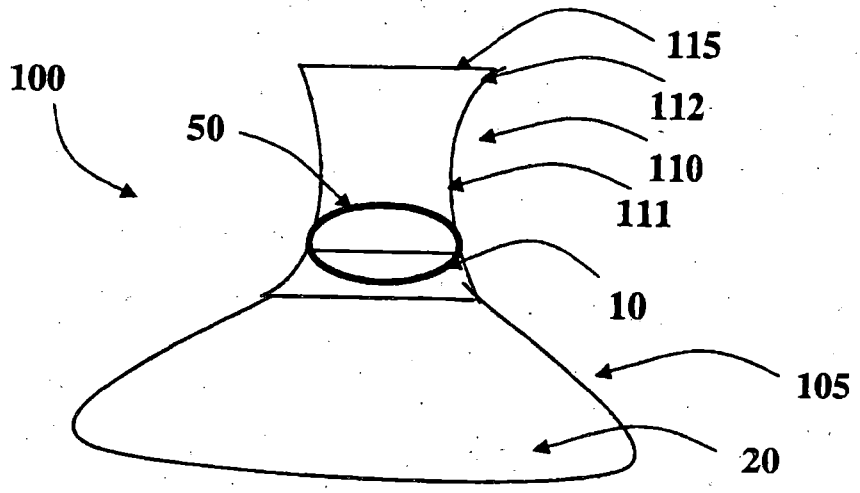

Fig. 13

Bottle with a Flow-impeding Device for Dispensing of Non-Uniform Liquids

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application 60/935,572, filed August 20, 2007, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to the field of bottles for containing and dispensing of liquids and more particularly, to bottles for containing and dispensing of non-uniform liquids.

BACKGROUND OF THE INVENTION

[0003] Currently, mouthwash bottles, as most other bottles for dispensing of liquids, have an aperture area, which is either equal or slightly smaller than the opening area of the edge part of the bottle that meets the aperture. This is mostly sufficient for dispensing liquids of a uniform phase. However, in the case of a two-phase mixture such as a two-phase mouthwash, which is a combined mouthwash for oral hygiene that is composed of two liquids of unequal specific-weight and viscosity, where one phase floats above the heavier phase - a too-large aperture may cause the upper-phase to escape too quickly when the bottle is near full leaving a smaller amount of the upper-phase for the next pour and so forth – until the proportions of the phases of the poured liquid are reversed, leaving hardly any of the upper-phase when the bottle is half full or near empty.

[0004] To use the two-phase liquid such as the two-phase mouthwash, which contains a two-phase mixture of oil and watery solution, for example, one mixes the phases (e.g. by shaking of the bottle), creating a temporary emulsion. Then the cap is removed and the liquid poured into a cap (e.g. the cap of the bottle). During the time elapsed (while the cap is removed and the bottle is turned over by the user for the pouring), the phases start to separate so that there is a top layer that is rich in oil and poor in water. Even if the cap is removed quickly, the hand mixing procedure usually results in a temporary emulsion that starts to separate right after mixing within a short period of time following the mixing (e.g. less than one second). This is particularly the case when the bottle is full, which makes the mixing more difficult.

[0005] The result is that when the bottle is full, the initial dispensing yields an oil-rich and water-poor mixture. When the consumption of the contents of the bottle is towards the end, the ratio reverses. In the case of the two-phase mouthwash the ratio of aqueous: oil phase is about 85:15 w/w, this means that at the beginning of use, the user may dispense a disproportionately large portion of the oil phase when the bottle is full or near full, resulting in less-than-desirable taste and function, which repeats itself in reverse when most of the contents of the bottle have been consumed, and the oil:aqueous ratio is lower than intended. It is the purpose of the Invention therefore to ensure a more proportionate delivery of the mixture.

SUMMARY OF THE INVENTION

[0006] The present invention, according to non-limiting embodiments thereof, provides a bottle for containing and dispensing of a non-uniform liquid such as a two-phase mouth wash, for instance.

[0007] The liquid may comprise two phases: a lower-phase and an upper-phase where the lower-phase is a liquid of a higher specific-weight than that of the upper-phase, which enables the upper-phase to naturally form above the top one.

[0008] According to some embodiments of the invention, the bottle may comprise a flow-impeding device, where the flow-impeding device impedes the flow of the liquid that is dispensed from the bottle to allow impeding the dispensing of an uppermost layer of the liquid such as at least a portion of the upper-phase.

[0009] The user may be required to mix the phases before pouring the liquid into a cap, to create a temporary emulsion of the two liquids, in which case the upper-phase may start reforming immediately after the mixing. The flow-impeding device may be used to impede the dispensing of the reforming upper-phase.

[0010] According to some embodiments of the invention, the bottle may comprise a body, which is the lower part of the bottle; a neck integrally connected to said body located above the body, wherein the top edge of said neck defines an opening of the bottle having a predefined circumference; and a flow-impeding device connected to said opening along the circumference of the neck.

[0011] According to some embodiments of the invention, the flow-impeding device comprises at least one protruding sealing member creating at least one reduced opening, wherein each protruding sealing member is a curved structure creating at least one cavity therein, enabling to receive the liquids from within the bottle.

[0012] The protruding sealing member may enable delaying the dispensing of the upper-phase when the bottle is positioned in a pouring tilted position (e.g. when the bottle is tilted and the protruding sealing member is situated above the reduced opening), by sealing at least part of the bottle's opening and by enabling at least part of the upper-phase to be contained by said cavity.

[0013] The liquid in the bottle may be dispensed into any suitable cap known in the art, such as for example a cup like structure that can also serve as the bottle's lid.

[0014] The body may have a trimmed conical shape having a bottom-base, which is wider than the top-base, wherein the circumference of the lower edge of the neck is equal to the circumference of the upper edge of the body.

[0015] According to some embodiments of the invention, the flow-impeding device may comprise two protruding sealing members connected to one another to create the reduced opening, where the reduced opening is mouth-shaped.

[0016] According to some embodiments of the invention, the bottle may further comprise a protruding ring, which is a circular protruding structure creating a ring cavity therein, wherein said ring integrally connects the upper edge of the body to the lower edge of the neck.

[0017] According to some embodiments of the invention, at least one of the protruding sealing members may comprise at least one hole enabling to enhance the flow rate of the liquid when it is dispensed by allowing air to enter the bottle while the exiting liquid dispensed therefrom, which may seal the reduced opening.

[0018] According to some embodiments of the invention, at least one of the protruding sealing members may additionally or alternatively comprise at least one indicator thereon, which enables indicating the right positioning of the sealing member when used for dispensing of said liquid. For example, the indicator may be an arrow sign indicating that the sealing member upon which it is positioned should be held upwardly when tilted to a pouring position – so that this indicated sealing member would be situated above the reduced opening when the user dispenses the liquid from the bottle.

[0019] According to other embodiments of the invention, the protruding sealing member may be a half sphere situated at the center of the bottle's opening separated from the circumference of the neck and connected thereto by securing means, creating reduced openings situated along a ring encircling the protruding sealing member.

[0020] According to some embodiments of the invention, the bottle's body may include at least one right-angled corner at the lower part of the bottle, where that corner is constructed from two crossing walls of the body and the body's base, where the walls are perpendicular to one another and to the base.

[0021] According to alternative embodiments of the invention, the bottle for containing and dispensing of a two-phase liquid may comprise a body, which is the lower part of the bottle; a neck integrally connected to said body located above the body, wherein the top edge of said neck defines an opening of the bottle having a predefined circumference; and at least one separate flow-impeding device inserted into said bottle. The separate flow-impeding device may be a piece that is separated from the bottle, where said separate flow-impeding device is of shape and dimensions that enable the neck opening to block the separate flow-impeding device from exiting the bottle, thereby enabling it to reduce the area of the bottle's opening and therefore to facilitate in impeding the dispensing of the upper-phase.

[0022] According to these alternative embodiments of the invention, the separate flow-impeding device enables changing of its dimensions to allow contracting when inserted into the bottle through the neck and retracting once inside the bottle.

[0023] The separate flow-impeding device may be made from any elastic material enabling it to change its dimensions according to pressure applied thereon such as, for example, rubber, elastic polymer-based materials and the like.

[0024] According to some embodiments of the invention, the separate flow-impeding device may be a ball like shape with retractable protrusions and niches, where the protrusions enable entering the niches once pushed through the neck and retracting back to an open protruding position once inside the bottle.

[0025] The specific-weight of the flow-impeding device may be either higher than that of the upper-phase and smaller than that of the lower-phase, allowing it to float between the two phases once the bottle opening faces upwards, or smaller than that of the upper-phase allowing it to float above both phases.

[0026] Additionally or alternatively, the separate impeding device that is inserted into the bottle may facilitate in dispersing of the two-phases and creating a substantially homogeneous emulsion of the two-phases once the liquid in the bottle is mixed.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0027] The subject matter regarded as the invention will become more clearly understood in light of the ensuing description of embodiments herein, given by way of example and for purposes of illustrative discussion of the present invention only, with reference to the accompanying drawings, wherein

Fig. 1 is a schematic illustration of a commonly used bottle containing a two-phase liquid;

Fig. 2 is a schematic illustration of a commonly used bottle containing a two-phase liquid in a pouring position;

Fig. 3 is a schematic illustration of a commonly used symmetric bottle containing a two-phase liquid in a different pouring position.

Fig. 4 is a schematic illustration of a bottle containing a two-phase liquid with a flow-impeding device, according to some embodiments of the invention;

Fig. 5 is a schematic illustration of a bottle containing a two-phase liquid with a flow-impeding device at a pouring position, according to some embodiments of the invention;.

Fig. 6 is a schematic illustration of a top view of a flow-impeding device connected to a bottle, according to some embodiments of the invention;

Fig. 7 is a schematic illustration of a top view of a flow-impeding device connected to a bottle, according to additional embodiments of the invention;

Fig. 8A and Fig 8B are schematic illustrations of a top and a side view respectively, of a bottle containing a two-phase liquid with a flow-impeding device, according to other embodiments of the invention;

Fig. 9 is a schematic illustration of a straight angular two-phase liquid bottle, according to some embodiments of the invention;

Fig. 10 is a schematic illustration of a side view of a bottle containing a two-phase liquid with a straight-angular body in a pouring position, according to some embodiments of the invention;

Fig. 11 is a schematic illustration of a side view of a bottle containing a two-phase liquid with a retractable-separated flow-impeding device, according to some embodiments of the invention;

Fig. 12A and Fig. 12B are schematic illustrations of a retractable separate flow-impeding device in an un-contracted state and in a contracted state respectively, according to some embodiments of the invention; and

Fig. 13 is a schematic illustration of a side view of a bottle containing a two-phase liquid with a separate flow-impeding device, according to other embodiments of the invention.

[0028] The drawings together with the description make apparent to those skilled in the art how the invention may be embodied in practice.

DETAILED DESCRIPTIONS OF SOME EMBODIMENTS OF THE INVENTION

[0029] The present invention, in some embodiments thereof, provides a flow-impeding device **150**, which may be jointly configured with a bottle **100** or a separate piece, where the bottle **100** may contain a non-uniform liquid having two phases, where the upper-phase **10** of the liquid is of a lower specific-weigh than that of the lower-phase **20**. For example, the upper-phase **10** may be a liquid made from an oily substance and the lower-phase **20** of a watery solution, as illustrated in Fig. 4.

[0030] Fig. 1, Fig. 2 and Fig. 3 schematically illustrate a commonly used bottle **100** that comprises a body **105** a protruding ring **120** and a neck **110** that is narrower than the body **105**, where the body **105**, the ring **120** and the neck **110** are jointly configured to create the bottle **100** and may be molded as a single piece at the production process.

[0031] The protruding ring **120** may create a cavity along the circumference of the upper part of the body **105** and the lower part of the neck **110** connecting them to one another.

[0032] The upper circumference **115** of the bottle **100** may define an opening of the bottle **100**. The bottle **100** may be sealed by a cap, which may also serve as the cap **200** of the liquid poured from the bottle **100** therein.

[0033] In this configuration, which is commonly used in the art, the initial dispensing of the two-phase liquid into the cap **200** yields a mixture which is rich in liquid of the upper-phase **10** (e.g. oil) and poor in liquid of the lower-phase **20**, where "poor" and "rich" relates to an exceeding ratio between the two phases **10** and **20**, where commonly the upper-phase **10** dispensed volume should be much smaller than the volume of the dispensed bottom phase **20**. When the contents of the bottle is

almost fully consumed or dispensed, the ratio reverses, since very little if any of the upper-phase **10** liquid remains in the bottle **100**.

[0034] As illustrated in Fig. 2, at a first pouring position in which, for example, the upper-phase **10** and the lower-phase **20** may both reach the opening of the bottle **100**, depending upon the volumes and volume ratios of the liquids in the bottle **100** and the shape and volume of the bottle **100** and opening.

[0035] Enlarging the pouring angle into a second pouring position as illustrated in Fig. 3, may facilitate in allowing the upper-phase **10** to naturally float upwards without reaching the bottle's **100** opening. Unfortunately, the pouring-angle depends upon the hand-movements of the user, which are hard to predict.

[0036] Narrowing of the bottle's **100** opening aperture circumference **115** using a flow-impeding device may facilitate in (a) allowing the natural floating feature of the upper-phase **10** to prevent it from over-spilling; and (b) facilitating in forcing or encouraging the user to enlarge the tilting angle of the bottle **100** in a pouring position to prevent the upper-phase **10** from reaching the opening or at least reduce the volume of the uppermost layer (e.g. a portion of a reformed upper-phase that has reformed after the mixing has been carried out) that reaches said reduced opening **151**.

[0037] When the liquid is shaken and mixed and becomes an emulsion, right before pouring, the upper-phase **10** reforms to some extent right after mixing. In that case, the bottom layer is the emulsion, which is a temporary mixture of the top and bottom phases **10** and **20**, where the uppermost layer contains a lower portion of the upper-phase **10**. The flow-impeding device **150** allows impeding the dispensing of that reformed upper-phase **10** and thereby facilitates in keeping the proportions between the top and bottom phases **10** and **20** substantially equal to their initial lower/upper phases-ratios throughout the use of the liquid in the bottle **100**.

[0038] While the description below contains many specifications, these should not be construed as limitations on the scope of the invention, but rather as exemplifications of the preferred embodiments. Those skilled in the art will envision other possible variations that are within its scope. Accordingly, the scope of the invention should be determined not by the embodiment illustrated, but by the appended claims and their legal equivalents.

[0039] An embodiment is an example or implementation of the inventions. The various appearances of "one embodiment," "an embodiment" or "some embodiments" do not necessarily all refer to the same embodiments. Although various features of the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

[0040] Reference in the specification to "one embodiment", "an embodiment", "some embodiments" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least one embodiments, but not necessarily all embodiments, of the inventions. It is understood that the phraseology and terminology employed herein is not to be construed as limiting and are for descriptive purpose only.

[0041] The principles and uses of the teachings of the present invention may be better understood with reference to the accompanying description, figures and examples. It is to be understood that the details set forth herein do not construe a limitation to an application of the invention. Furthermore, it is to be understood that the invention can be carried out or practiced in various ways and that the invention

can be implemented in embodiments other than the ones outlined in the description below.

[0042] It is to be understood that the terms "including", "comprising", "consisting" and grammatical variants thereof do not preclude the addition of one or more components, features, steps, or integers or groups thereof and that the terms are to be construed as specifying components, features, steps or integers. The phrase "consisting essentially of", and grammatical variants thereof, when used herein is not to be construed as excluding additional components, steps, features, integers or groups thereof but rather that the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

[0043] If the specification or claims refer to "an additional" element, that does not preclude there being more than one of the additional element. It is to be understood that where the claims or specification refer to "a" or "an" element, such reference is not be construed that there is only one of that element. It is to be understood that where the specification states that a component, feature, structure, or characteristic "may", "might", "can" or "could" be included, that particular component, feature, structure, or characteristic is not required to be included.

[0044] Where applicable, although state diagrams, flow diagrams or both may be used to describe embodiments, the invention is not limited to those diagrams or to the corresponding descriptions. For example, flow need not move through each illustrated box or state, or in exactly the same order as illustrated and described.

[0045] Methods of the present invention may be implemented by performing or completing manually, automatically, or a combination thereof, selected steps or tasks. The term "method" refers to manners, means, techniques and procedures for

accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the art to which the invention belongs. The descriptions, examples, methods and materials presented in the claims and the specification are not to be construed as limiting but rather as illustrative only.

[0046] Meanings of technical and scientific terms used herein are to be commonly understood as by one of ordinary skill in the art to which the invention belongs, unless otherwise defined. The present invention can be implemented in the testing or practice with methods and materials equivalent or similar to those described herein.

[0047] Any publications, including patents, patent applications and articles, referenced or mentioned in this specification are herein incorporated in their entirety into the specification, to the same extent as if each individual publication was specifically and individually indicated to be incorporated herein. In addition, citation or identification of any reference in the description of some embodiments of the invention shall not be construed as an admission that such reference is available as prior art to the present invention.

[0048] Fig. 4 and Fig. 5 schematically illustrate a bottle **100** for containing and pouring of a non-uniform two-phase liquid comprising an upper-phase **10** and a lower-phase **20** liquids, where the upper-phase **10** is lighter (having a lower specific weight) than the lower-phase **20**, according to some embodiments of the invention.

[0049] According to some embodiments of the invention, the non-uniform liquid may be a two-phase mouthwash containing substances that can be used for mouthwash, where each phase may have a different function in desorbing bacteria in a

user's oral tissue for mouth hygiene, such as the two phase liquids described in a US Patent No. 6465521, which is incorporated by reference herein in its entirety.

[0050] According to these embodiments, as illustrated in 4 and Fig. 5, the bottle **100** may comprise:

- a body **105**, which may have a trimmed conical shape having a bottom-base which is wider than the top-base;
- a neck **110**, which may be equal in diameter to the top-base of the body **105**, where the top edge of the neck creates an opening with a predefined circumference **115**;
- a protruding ring **120**, which may encircle the bottom part of the neck **110** connecting it to the body **105**, creating an enlarged cavity area encircling the connecting area between the neck **110** and the body **105**; and
- a flow-impeding device **150** connected to the top edge of the neck **110** along the circumference **115** comprising at least one protruding sealing member **152**, which may be curved structures creating at least one cavity **30** therein, where the member(s) **152** create a reduced opening **151**, where each sealing member may be a curved structure creating at least one cavity **30** therein.

[0051] The protruding sealing-members **152** may be jointly configured with the bottle **100**, and may integrally connect to the top edge of the neck **110** along its upper circumference **115**. Additionally, the protruding sealing-members **152** may connect to one another to create the reduced opening **151**, where the area of the reduced opening **151** is smaller than the area of the original opening created by the neck's **110** circumference **115**.

[0052] Once the protruding sealing member **152** is positioned upwards in a pouring tilted position, as illustrated in fig. 5, the lighter upper-phase liquid **10** may be

delayed, since some of it may be caught in the cavity **30** of the upper protruding member **152**. The reduced opening **151** may delay the exit of the entire liquid, by slowing down the pouring flow rate; therefore allow a portion of the upper-phase **10** to exit the bottle **100** only once enough of the lower-phase **20** is already dispensed.

[0053] Additionally, a lower protruding sealing member **152** may allow further impeding the exit of the heavier lower-phase liquid **20** to prevent it from pouring too fast ending up with an exceeding portion of the lower-phase **20** at the cap **200**. Since the quantity (e.g. in volume units) of the upper-phase **10** may be smaller than the quantity of the lower-phase liquid **20** when the bottle **100** is full and unused, the additional delaying of the lower-phase liquid's **20** exiting may be desirable.

[0054] Additionally, the user may be required to shake the bottle **100** before pouring so as to create the emulsion mixture of the two phases where only a fraction of the upper-phase **10** reforms on the upper layer above the emulsion. The dispensing of the remaining upper-phase **10** may be delayed or even prevented by the upper protruding sealing member **152** thereby ensuring the dispensing of a liquid comprising mostly or only the emulsion, which contains the right pre-calculated recommended ratio between the two phases **10** and **20**.

[0055] Additionally, once the flow-impeding device **150** delays or blocks some of the upper-phase **10** from exiting through the reduced opening **151** (depending on the pouring angle at which the bottle is tilted for the dispensing of the liquid), the upper-phase's **10** impact against the inner walls of the flow-impeding device **150**, when tilting the bottle **100**, may further facilitate in throwing the upper-phase **10** backwards away from the reduced opening **151**, thereby further enhance the delaying effect.

[0056] The design of the protruding sealing-members **152** may also enhance the delay effect, since the cavity **30** or cavities **30** created by the protrusions may allow a containing space in which the liquid can linger during the pouring time interval.

[0057] Fig. 6 and Fig. 7 schematically illustrate top views of two possible designs for the flow-impeding device **150**, according to embodiments of the invention. The flow-impeding device **150** may be created by two protruding sealing-members **152** that are connected in a configuration that creates the reduced opening **151** with a mouth-like shape, as illustrated in fig. 7, or any other shape e.g. as illustrated in fig. 6.

[0058] Each sealing-member **152** may additionally include at least one small hole **154**, to slightly enhance the flow rate of the liquids from the bottle **100**, so that the pouring of the liquid will not become too slow due to the reducing of the bottle's **100** opening.

[0059] As known in the art, allowing more air pressure from outside the bottle **100** to enter the bottle **100** may facilitate in accelerating the exit of the liquid from the bottle **100**. The two competing effects' (delaying and acceleration) elements may be designed to allow an easy and comfortable pouring while keeping the phases ratios when dispensing the liquid as desirable as possible throughout the usage of the liquid.

[0060] Additionally, as illustrated in Fig. 6, at least one of the protruding sealing members **152** may further comprise an indicator **158** marked (e.g. curved thereon, printed thereon and the like) on the outer surface of the protruding sealing member **152**. The indicator **158** may indicate the right way(s) of holding the bottle **100** when pouring to allow the protruding sealing member **152** to point upwards when dispensing the liquid and thereby to optimally delay the dispensing of the upper-phase **10**.

[0061] According to some embodiments of the invention, the flow-impeding device **150** may include a multiplicity of reduced openings **151** to create a more symmetrical structure of the reduced openings **151** and of the sealing members **152** so that the pouring rotational angle of the bottle **100** may result in substantially the same effect. Alternatively, if the reduced opening **151** position and/or shape is asymmetric, the user may be required to hold the bottle **100** according to predefined rotation angle(s) that may be marked upon the bottle **100**. For example, in the embodiment illustrated in Fig. 6, there is a polar symmetry (the bottom side is a mirror view of the top side of the flow-impeding device **150**), therefore the user may be required to hold the bottle **100** in two possible pouring rotation angles.

[0062] Fig. 8A and Fig. 8B schematically illustrate the flow-impeding device **150** according to additional embodiments of the invention. A protruding circular sealing-member **152** may be seated substantially at the center of the circumference **115** of the bottle's **100** edge. The protruding sealing member **152** may be half a sphere fastened to the circumference **115** of the neck **110** by any securing means **155** known in the art, where the securing means **155** enable separating the protruding sealing member **152** from the circumference **115** of the neck **100** thereby creating reduced openings **151** situated along a ring encircling the protruding sealing member **152**.

[0063] Once tilted into a pouring position – the upper-phase **10** may reach the cavity **30** inside the protruding sealing-member **152** delaying the dispensing of the upper-phase **10**, while the lower part of the ring-like reduced opening **151** enables the lower-phase **20** to exit and the upper part of the reduced opening **151** serves as a hole or holes enabling to slightly enhance the dispensing of the lower-phase **20** liquid.

[0064] The bottle **100** may be made of any solid material known in the art or a collection or mixture of several materials such as plastic, glass, metal and the like.

[0065] Fig. 9 and Fig. 10 schematically illustrate an alternative bottle **100** shape that may also facilitate in the delaying effect. At least one of the side corners at the lower part of the bottle **100** may be perpendicular to the bottle's **100** base, where the two crossing walls of the body **110** creating the corner are also perpendicular to one another. This right angled corner may enhance the flow of the lower-phase **20** towards the opening of the bottle **100** by leaving less space for the lower-phase **20** to remain in once the liquid is poured with the right-angled corner facing downwards, as illustrated in Fig. 10.

[0066] Fig. 11, Fig. 12A and Fig. 12B may schematically illustrate a separate apertures reducer **50**, according to alternative embodiments of the invention. According to these embodiments, the separate flow-impeding device **50** may be a separated piece inserted into the bottle **100** in order to enable sealing some of the bottle's **100** opening when the two-phase liquid is poured by enabling to move inside the bottle **100**. The separate flow-impeding device **50** may be made of a material that may float above the upper-phase **10** (meaning that the flow-impeding device **50** is of a specific weight that is lower than that of the upper-phase **10**), or above the lower-phase **20** between the two phases as illustrated in Fig. 10 (meaning that the flow-impeding device **50** is of a specific weight that is higher than that of the upper-phase **10** and lower than that of the lower-phase **20**). The neck **110** opening may enable blocking the flow-impeding device **50** by having a smaller diameter than the diameter of the separate flow-impeding device **50**.

[0067] To be able to insert the separate flow-impeding device **50** into the bottle **100** the separate flow-impeding device **50** may be retractable enabling to enlarge its dimensions once inserted into the bottle **100**. For example, the separate flow-impeding device **50** may be a ball like shape with retractable protrusions **52** inserted into niches

53, where the protrusions **52** can retract to an open protruding position (as illustrated in Fig. 12A). Once the user and/or the manufacturer pushes the separate flow-impeding device **50** into the bottle **100** through the neck **110**, the protrusions **52** that are in contact with the neck **110** may be pushed into their niches **53** (as illustrated in Fig. 12B), thereby diminish the flow-impeding device's **50** dimensions allowing it to be inserted into the bottle **100**. Once inside the bottle **100**, where no substantial strain is applied upon the separate flow-impeding device **50**, the retractable protrusions **52** may pop out again and remain in that position while inside the bottle **100**. In a retracted position, the separate flow-impeding device **50** may block some of the opening of the neck **110**, since it may fall into the bottle **100** opening along with the liquid that is being dispensed and since in a retracted position the separate flow-impeding device **50** is of bigger dimensions than the diameter of the opening it may enable reducing the area of the opening and thereby impeding the dispensing of the upper-phase **10**.

[0068] Alternatively, the retractable separate flow-impeding device **50** may be made of retractable elastic materials such as rubber or elastic polymers, enabling to change its dimensions by contracting when being squeezed through the neck **110** and retracting once no pressure is applied thereon.

[0069] Depending on embodiments of the invention, the bottle **100** may comprise a multiplicity of separate flow-impeding devices **50** of sizes and shapes according to the bottle **100** size, shape and other practical and/or advertising considerations of the manufacturer.

[0070] The separate flow-impeding device (s) **50** may additionally facilitate in dispersing of the mixture of phases once the two phases are mixed and thereby

facilitate in creating a substantially homogeneous emulsion of the two-phases once the liquid in the bottle **100** is mixed.

[0071] According to some embodiments of the invention, the user may be required to mix the phases **10** and **20** (e.g. by shaking the bottle **100**) to create the emulsion, and then to pour a portion of the liquid into the cap **200**. A new thinner upper-phase **10** may reform again at the upper surface above the emulsion, during the time interval that has passed between the mixing and the pouring, which may be at least partially impeded in the bottle **100** by the flow-impeding device **150**.

[0072] Fig. 13 is a schematic illustration of a side view of a bottle **100** containing a two-phase liquid with a separate flow-impeding device **50**, according to other embodiments of the invention. According to these embodiments, the lower part **111** of the neck **110** may be situated between the upper part of the body **105** and the upper part of the neck **112**. The encircling walls of the lower part **111** may be narrower (in aperture) than the encircling walls of the upper part **112** of the neck **110** and the upper part of the body **105**. The narrower lower part **111** of the neck **110** may enable holding the flow-impeding device **50** therein, preventing the device **50** from either falling into the cavity of the body **105** or exiting the bottle from the opening, when the bottle **100** is tilted into a pouring position.

[0073] The separate flow-impeding device **50** may be a rounded device that is retractable enabling to change its size to be inserted into the bottle through the bottle's opening **115** and retracting back into a larger size once inside the narrowed area **400**. The walls of the upper part **111** may be narrower than at least one of the diagonals of a cross section area of the flow-impeding device **50**. Therefore, once the bottle **100** is tilted into a pouring position, the flow-impeding device **50** is trapped inside the

narrow lower part **111** of the neck **110** enabling blocking a part of the bottle's **100** opening and thereby impeding the flow of the dispensed liquid.

[0074] While the invention has been described with respect to a limited number of embodiments, these should not be construed as limitations on the scope of the invention, but rather as exemplifications of some of the preferred embodiments. Those skilled in the art will envision other possible variations, modifications, and applications that are also within the scope of the invention. Accordingly, the scope of the invention should not be limited by what has thus far been described, but by the appended claims and their legal equivalents.

What is claimed is:

1. A bottle for containing and dispensing of a non-uniform liquid, wherein said bottle comprises a flow-impeding device, wherein said flow-impeding device impedes the flow of the liquid that is dispensed from the bottle to allow impeding the dispensing of the liquid uppermost layer, by reducing the aperture of the bottle's opening.

2. The bottle of claim 1, wherein said non-uniform liquid is a two-phase liquid having a lower-phase liquid and an upper-phase liquid, where the specific weight of the lower-phase liquid is higher than the specific weight of the upper-phase liquid, wherein said upper-phase naturally floats above said lower-phase.

3. The bottle of claim 1 further comprising

   - a body, which is the lower part of the bottle; and
   - a neck integrally connected to said body located above the body, wherein the top edge of said neck defines an opening of the bottle having a predefined circumference,

wherein said flow-impeding device is integrally connected to the circumference of the bottle's opening, and

wherein said device comprises at least one protruding sealing member creating at least one reduced opening, wherein said protruding sealing member is a curved structure creating at least one cavity enabling to receive the liquids from within the bottle,

wherein said member enables impeding the dispensing of the uppermost layer when the bottle is positioned in a pouring tilted position by sealing a part of the bottle's opening and by enabling at least part of the uppermost layer to be contained by said cavity.

4. The bottle of claim 3, wherein said impeding is enabled once said at least one protruding sealing member is located above the reduced opening in the pouring position.

5. The bottle of claim 3, wherein said flow-impeding device comprises two protruding sealing members connected to one another to create the reduced opening.

6. The bottle of claim 3, wherein said flow-impeding device comprises two protruding sealing members connected to one another to create the reduced opening.

7. The bottle of claim 6, wherein said two protruding sealing members are connected to one another in a configuration that creates a mouth-shaped reduced opening.

8. The bottle of claim 1 further comprises a cap, which serves as a container for containing of the dispensed liquid and as a lid of the bottle.

9. The bottle of claim 3, wherein at least one of the protruding sealing members comprises at least one hole enabling to enhance the flow rate of the liquid that is dispensed.

10. The bottle of claim 3, wherein at least one of the protruding sealing members comprises at least one indicator thereon, which enables indicating the correct positioning of the sealing member when used for dispensing of said liquid.

11. The bottle of claim 3, wherein said protruding sealing member is rounded and situated at the center of the bottle's opening separated from the circumference of the neck and connected thereto by securing means, creating reduced openings situated along a ring encircling the protruding sealing member.

12. The bottle of claim 1 further comprising a protruding ring, which is a circular protruding structure creating a ring cavity therein, wherein said ring integrally connects the upper edge of the body to the lower edge of the neck.

13. The bottle of claim 1, wherein the non-uniform liquid is a two-phase mouthwash containing substances that can be used for mouthwash, wherein each phase may have a different function in desorbing bacteria in a user's oral tissue.

14. The bottle of claim 3, wherein the body has a trimmed conical shape having a bottom-base which is wider than the top-base, wherein the circumference of the lower edge of the neck is equal to the circumference of the upper edge of the body.

15. The bottle of claim 3, wherein said body includes at least one right-angled corner at the lower part of the bottle, wherein said corner is constructed from two crossing walls of the body and the body's base, where the walls are perpendicular to one another and to the base.

16. The bottle of claim 1, further comprising:

- a body, which is the lower part of the bottle;
- a neck integrally connected to said body located above the body, wherein the top edge of said neck defines an opening of the bottle having a predefined circumference; and
- at least one separate flow-impeding device inserted into said bottle,

wherein said separate flow-impeding device is a piece that is separated from the bottle,

wherein said separate flow-impeding device is of shape and dimensions that enable the neck opening to block the separate flow-impeding device from exiting the bottle, thereby enabling the separate flow-impeding device to reduce

the area of the bottle's opening and therefore to facilitate in impeding the dispensing of the upper-phase.

17. The bottle of claim 16, wherein said separate flow-impeding device enables changing of its dimensions to allow contracting when inserted into the bottle through the neck and retracting once inside the bottle.

18. The bottle of claim 17 wherein the lower part of the neck is situated between the upper part of the body and the upper part of the neck, wherein the encircling walls of the lower part are narrower than the encircling walls of the upper part of the neck 1and the upper part of the body,

Wherein the narrower lower part of the neck enables holding the flow-impeding device therein, preventing the device from either falling into the cavity of the body or exiting the bottle from the opening, when the bottle is tilted in a pouring position,

wherein the walls of the upper part of the neck are narrower than at least one of diagonal of the flow-impeding device, therefore, the narrow upper part enables trapping the flow-impeding device therein to block a part of the bottle's opening and thereby impede the flow of the dispensed liquid.

19. The bottle of claim 17, wherein said separate flow-impeding device is made from elastic materials enabling it to change its dimensions according to pressure applied thereon.

20. The bottle of claim 17, wherein said separate flow-impeding device is round, with retractable protrusions and niches, wherein the protrusions enable entering the niches once pushed through the neck and retracting back to an open protruding position once inside the bottle.

21. The bottle of claim 16, wherein the specific-weight of the flow-impeding device is higher than that of the upper-phase and lower than that of the lower-phase, wherein the separate flow-impeding device floats between the two phases once the bottle opening faces upwards.

22. The bottle of claim 16, wherein the specific-weight of the upper-phase is lower than that of the flow-impeding device, wherein the separate flow-impeding device floats above the upper-phase once the bottle opening faces upwards.

23. A method of dispensing a non-uniform liquid from a bottle containing said liquid, wherein said non-uniform liquid comprises a lower-phase and an upper-phase where the lower-phase is a liquid of a higher specific-weight than the upper-phase, said method comprising:

mixing of the two-phases creating a temporary emulsion; and

pouring a portion of the liquid into a cap,

wherein said flow-impeding device impedes the flow of the liquid that is dispensed from the bottle to allow the dispensing of a reformed upper-phase from said bottle.

24. The method of claim 20 further comprising the step of receiving at least a portion of the reformed upper-phase,

wherein said flow-impeding device comprises at least one protruding sealing member creating at least one reduced opening, wherein said protruding sealing member is a curved structure creating at least one cavity therein enabling to receive the liquids from within the bottle,

wherein said member enables impeding the dispensing of the upper-phase when the bottle is positioned in a pouring tilted position by sealing at least part of the

bottle's opening and by enabling at least part of the reformed upper-phase to be contained by said cavity.

25. The method of claim 20, wherein said impeding is carried out by sealing a part of the bottle's opening, wherein said flow-impeding device is a separate piece that is separated from the bottle and is inserted into the bottle,

wherein said separate flow-impeding device enables moving inside the bottle, wherein the opening of said bottle blocks it from exiting the bottle, thereby enabling the separate flow-impeding device to reduce the area of the bottle's opening and therefore to facilitate in impeding the dispensing of the reformed upper-phase.

26. A bottle for containing and dispensing of a non-uniform liquid comprising a lower-phase and an upper-phase where the lower-phase is a liquid of a higher specific-weight than the upper-phase, wherein said bottle comprises a body, which is the lower part of the bottle; and a neck integrally connected to said body located above the body, wherein the top edge of said neck defines an opening of the bottle

wherein said body includes at least one right angled corner at the lower part of the bottle, wherein said corner is constructed from two crossing walls of the body and a base of the body, wherein the walls are perpendicular to one another and to the base to allow impeding of the dispensing of the top phase by enhancing the flow of the lower-phase towards the opening of the bottle by leaving less space for the lower-phase to remain in, once the liquid is poured with the right-angled corner facing downwards.

27. The bottle of claim 26 wherein said two-phase liquid is a mouthwash containing substances that can be used for mouthwash, wherein each phase may have a different function in desorbing bacteria in a user's oral tissue.

28. A bottle for containing and dispensing of a non-uniform liquid comprising of an upper-phase and a lower-phase, wherein the upper-phase has a lower specific weight than that of the lower-phase, said bottle further comprising at least one separate device that is inserted into said bottle, wherein said device facilitates in dispersing of the two-phases and creating a substantially homogeneous emulsion of the two-phases once the liquid in the bottle is mixed.

29. The bottle of claim 28, wherein said separate device enables changing of its dimensions to allow contracting when inserted into the bottle through the neck and retracting once inside the bottle.

30. The bottle of claim 28, wherein said separate device is made from elastic materials enabling it to change its dimensions according to pressure applied thereon.

31. The bottle of claim 28, wherein said separate device is round, with retractable protrusions and niches, wherein the protrusions are pushed into the niches once the device is pushed through the neck and retract back to an open protruding position once inside the bottle.

UK Intellectual Property Office

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Application No: GB0815230.8 **Examiner:** Mr Michael Prior

Claims searched: 1-22, 24, 25 **Date of search:** 29 October 2008

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-8, 12-14	WO 2004/103855 A1 (LANGELUND) See whole document
X	1, 2, 8, 12, 13	US 4187964 A (BOGART) See whole document
X	1, 2, 8, 12, 13	US 6085949 A (ZIMNY) See whole document
X	1, 2, 8, 12, 13	US 6367670 B1 (WARNER) See whole document
X,P	1, 2, 8, 12, 13	JP 2008087830 A (SHIBATA) See figures and abstract

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

B65D

The following online and other databases have been used in the preparation of this search report

Online: EPODOC, WPI

International Classification:

Subclass	Subgroup	Valid From
B65D	0001/02	01/01/2006

30

Subclass	Subgroup	Valid From
B65D	0006/02	01/01/2006
B65D	0023/04	01/01/2006

File: 2010-us-patent-7727515.pdf

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America, and if the invention is a process, of the right to exclude others from using, offering for sale or selling throughout the United States of America, or importing into the United States of America, products made by that process, for the term set forth in 35 U.S.C. 154(a)(2) or (c)(1), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b). See the Maintenance Fee Notice on the inside of the cover.

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If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the United States Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

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If the application for this patent was filed on or after June 8, 1995, the term of this patent begins on the date on which this patent issues and ends twenty years from the filing date of the application or, if the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121, or 365(c), twenty years from the filing date of the earliest such application ("the twenty-year term"), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b), and any extension as provided by 35 U.S.C. 154(b) or 156 or any disclaimer under 35 U.S.C. 253.

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US007727515B2

(12) **United States Patent**
Rosenberg Nevo

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(54) **DEODORANT COMPOSITIONS AND METHOD**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 929 days.

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(52) **U.S. Cl.** **424/65**; 424/401

(58) **Field of Classification Search** 424/401, 424/65, 76.3

See application file for complete search history.

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(57) **ABSTRACT**

A method for preventing the formation and/or for the removal of body odors, comprising priming the skin with a deodorant composition comprising an oil, for a period of time sufficient to allow binding of skin microorganisms and dissolution of odor into said composition, and thereafter washing off said composition with conventional detergents.

7 Claims, No Drawings

DEODORANT COMPOSITIONS AND METHOD

This application is a continuation of application Ser. No. 09/519,255, filed Mar. 6, 2000 now abandoned, which application(s) are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to deodorizing compositions. More particularly, the invention relates to a method for removing body odor and to deodorizing compositions for this purpose.

BACKGROUND OF THE INVENTION

Body odor is formed when fresh perspiration, which is odorless per se, is decomposed by microorganisms. This process takes place principally, though not solely, in the axilla, and a number of microorganisms are involved, each having different activity and leading to body odor of different strength and unpleasantness. The most prominent odor-producing microorganisms include aerobic diphtheroids, primarily *Corynebacterium* species and coagulase negative cocci such as Micrococcaceae.

The various microorganisms are found in different proportions in different individuals, and this is a reason for the fact that different individuals exhibit different body odors.

The commercial cosmetic deodorants are based on different active principles. The formation of perspiration is suppressed according to the known art by astringents, predominantly aluminum salts such as aluminum hydroxychloride. Apart from the denaturation of the skin proteins, however, the substances used for this purpose clog the pores, interfere drastically with the heat regulation of the axillary region, may cause cancer and other diseases, and should at best be used in exceptional cases. According to another accepted prior art method, the bacterial flora on the skin is reduced by antimicrobial substances. Ideally here, only the odor-causing microorganisms should be destroyed. In practice, however, it turns out that the non-odorous microflora of the skin, which may have a beneficial role, are damaged to the same extent. Finally, body odor can also be concealed by fragrances, which, however, is the least able to meet the aesthetic needs of the consumer, as the mixture of body odor and perfume fragrance smells rather unpleasant.

According to a recent patent on this subject (U.S. Pat. No. 5,318,778), deodorants should fulfill the following conditions:

1) The biological processes of the skin must not be impaired.

2) The deodorants should have no distinct intrinsic odor.

3) They must be harmless in the case of overdosage or other unintended use.

4) They should not concentrate on the skin after repeated use.

It should be possible to incorporate them easily into commercial cosmetic formulations.

Those which are known and usable are both liquid deodorants, for example aerosol sprays, roll-ons and the like and solid preparations, for example deodorant sticks, powders, powder sprays, intimate cleansers etc.

U.S. Pat. No. 5,318,778 approaches the problem by employing lantibiotics, which are said to be specific microbiocides which predominantly destroy odor-forming microorganisms.

All the prior art methods suffer from severe drawbacks: they require the masking of body odor which has already formed prior to the application of the deodorant, because the destruction of axillary microorganisms does not remove already formed odor. They require the use of antimicrobial agents which must inhibit the high microbial load and, quite importantly, they very often leave unpleasant stains or halos on the cloths, particularly at and around the axilla. Also the safety of many antiperspirants is dubious, due to the presence of potentially harmful components, and the result is often unpleasant.

It has now been surprisingly found, and this is an object of the invention, that it is possible to obviate all the aforesaid disadvantages of the prior art, in a simple and convenient way.

It is an object of the invention to provide a method for preventing and/or treating body odor, which is simple and convenient to use, and which does not leave a macroscopic residual layer on the skin.

It is another object of the invention to provide such a method, which does not lead to staining or haloing of clothing around the treated area.

It is yet another object of the invention to provide an anti-body odor composition, which is convenient and easy to use, and which does not leave unpleasant left-over results, such as staining or haloing materials.

The invention is, of course, primarily concerned with the prevention of body odors in humans, more particularly where such body odor formation is the most pronounced, i.e., in the axilla. However, the invention is by no means limited to the use in the axilla, and any other body part requiring treatment can be treated according to the invention. Additionally, the invention is suitable for use in subjects other than humans, e.g., in house pets.

Other objects and advantages of the invention will become apparent as the description proceeds.

SUMMARY OF THE INVENTION

In one aspect, the invention is directed to a method for preventing the formation and/or for the removal of body odors, comprising bringing into contact with the area to be treated a stable skin deodorizing composition comprising an oil, for a period of time sufficient to allow dissolution of skin microorganisms and odor into said composition, thus priming the skin so that when the skin is subsequently washed with conventional detergents, the odors and microorganisms are removed.

In the context of the invention, the term "dissolution of skin microorganisms and odor" should be interpreted as meaning that microorganisms and odors which normally adhere to the skin interact with the oil-containing composition, so that they are efficiently washed off the skin upon subsequent rinsing with soap and water. The actual mechanism by which such microorganisms and odors are removed from the skin by this procedure, and the processes that the microorganisms undergo by the action of the oil component, are not a concern of the present invention. It is sufficient to say that such microorganisms and odors are not easily detached from the skin and, for instance, they are not effectively removed by water and soap. This is the reason why regular detergents which are not harmful to the skin cannot efficiently counter body odor. However, once the body-odor forming microorganisms and odors have been efficiently removed from the skin and washed away, their fate is not important, as the primary object of the invention has been achieved.

As stated, after the application of the composition of the invention, and after some time has been allowed for the

microorganisms and odor to interact with said composition, and for the odor to solubilize into it, the composition can be washed away by conventional body detergents. This is conveniently done in the shower. Thus, little substantial residual materials are left on the skin—and there is less need to leave any such residual materials, since the effect of the invention, i.e., the removal of the microorganisms and odors has already been achieved.

The time needed for effectively priming the skin with the present compositions varies from one composition to the other, and will further vary from one person to the other. Each individual will easily determine the optimal time for an efficient and long-lasting treatment, but such times are generally in the range of 5 seconds to 5 minutes.

In another aspect, the invention is directed to a deodorizing composition comprising an oil as a skin priming agent for subsequent removal by rinsing with soap and water. The deodorizing composition can be provided in a variety of forms, e.g., as organic solutions and lotions. According to a preferred embodiment of the invention, the deodorizing composition of the invention is provided in the form of a solution and a stable water in oil or oil in water emulsion. The deodorizing composition according can further comprise additives, such as conventional deodorant components. While such additives are not essential to the invention, they may have collateral beneficial effects, such as residual scent, and their incorporation in the compositions of the invention is not deleterious to the invention. Such conventional deodorant components may comprise antibacterial and antiodor materials. Various different antibacterial and antiodor materials may be used in the invention. According to a preferred embodiment, however, the antibacterial material comprises benzalkonium chloride. As the compositions of the present invention do not remain in contact with the skin for long periods of time, the aforementioned antibacterial and antiodor materials may be used at concentrations higher than normally used in conventional deodorant preparations that remain on the skin for long periods of time. One such material Triclosan, may be used at concentrations of only 0.03 to 0.3% when used in conventional deodorants, but at higher levels (0.2 to 0.5%) when the composition is washed off (e.g. in the case of solid soap [Antiperspirants and Deodorants Ed. K Laden & C. Felger, 1988]). In the present invention, Benzalkonium chloride, for example, may be used at concentrations higher than 0.1% (w/v), a preferred range being 0.1% to 4.0% (w/v).

The oil to be used in the compositions of the invention may be any oil which primes the skin to enhance removal of bacteria and odors on subsequent soaping and washing. The skilled person will easily select suitable oils or oil combinations which give the desired results, by means of simple and straightforward tests. In principle, it is sufficient to rub the oil to be tested into the axilla of a subject. After waiting 3 minutes, the subject showers in the usual way, and an odor judge determines whether there has been a reduction in body odor, thus indicating whether the specific oil or oil combination is suitable for the intended purposes. Without wishing to limit the kind of oil employed in any way, it can be mentioned that illustrative and non-limitative examples of suitable oils include vegetable and synthetic oils, silicone based oils, isopropyl esters, hydrocarbons and their derivatives, or mixtures of two or more such oils. Suitable isopropyl esters are, for example, isopropyl myristate and isopropyl stearate.

Illustrative and non-limitative examples of suitable vegetal and animal oils include, e.g., sweet-almond oil, groundnut oil, wheatgerm oil, linseed oil, jojoba oil, apricot stone oil, walnut oil, palm oil, pistachio nut oil, sesame oil, rapeseed oil, cade oil, maize germ oil, peach stone oil, poppy seed oil, pine oil, castor oil, soya oil, avocado oil, safflower oil, coconut oil, hazelnut oil, olive oil, grapeseed oil, sunflower seed oil, whale oil, lard oil, horsehoof oil, tuna oil, caballine oil, otter oil, egg oil, sheep oil, seal oil, turtle oil, halibut liver oil, marmot oil, cod liver oil, neat's-foot oil and carbon oil. The oily phase can also contain a synthetic oil preferably consisting of carbon, hydrogen and oxygen, such as the glycol ethers or esters or glycerol ethers or esters described in French Patents No. 74/09,657, No. 75/24,656 and Nos. 75/24,657 and 75/24,658. The oily phase can also contain a mineral oil such as vaseline oil (liquid petrolatum), silicon oils or long-chain alcohols such as cetyl alcohol or stearyl alcohol.

When the composition of the invention is provided in the form of a stable emulsion, the emulsion may comprise water and/or a skin-compatible alcohol, and/or some water soluble components like propylene glycol, glycerol, thickeners, antibacterial materials etc. The final preparation can be in the form of a liquid, gel, cream, lotion, ointment or any other form suitable for body application.

Said emulsion can be a water-in-oil or an oil-in-water emulsion. Stable emulsions can be obtained using suitable conventional emulsifiers, such as sodium cetostearyl sulfate (Lanette E), polyoxyethylene cetyl/stearyl ether (Ceteareth-30) and polyoxyethylene laurylether (Dehydrol LS 3).

As stated, the composition of the invention is eventually almost entirely washed off the skin, although some small residue, which may remain on the skin, has no adverse effect. While this can be effected in any suitable way, it has been found that the deodorizing compositions according to the invention are particularly convenient for use as a pre-shower or in-shower deodorant. The invention is specifically intended to cover also pre-shower or in-shower deodorizing compositions comprising an oily phase.

The invention also encompasses a deodorizing kit comprising, in combination, any of the above-described deodorizing compositions, alongside a detergent. The detergent included in the kit may be in any convenient form, but preferably in the form of a solid or a liquid, or in the form of a gel or the like.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The above and other characteristics and advantages of the invention will be more readily apparent through the following detailed description of preferred embodiments thereof.

EXAMPLE 1

The assay of the antimicrobial activities of the various formulations was used as a rapid, primary screening method for identifying those formulations that are potentially useful as deodorants. Antimicrobial activity was tested by applying samples (5 µl) of the formulations (listed in Table I) onto lawns of axillary and other bacteria. (The liquid was applied directly to the agar.) Each active material of Table I was formulated in isopropyl myristate, at the concentration (% w/w) given in that table.

The effect of the formulations on microbial growth is shown in Table I below. In the table, "+" indicates full growth inhibition (transparent zone), "–" indicates lack of inhibition, and "+/–" indicates partial inhibition (translucent zone). The values (in centimeters) indicate the diameter of the inhibition zone.

The abbreviations used herein are as follows:

TAGAT: polyoxyethylene hydrogenated castor oil

IRGASAN: trichlorohydroxydiphenyl ether

TABLE I

PS Form No.	ACTIVE COMPONENTS	Bacillus	*S. epidermidis*	*S. aureus*	*C. xerosis*	*Micrococcus luteus*	*Serratia marcescens*
1	1% Benzalkanium chloride (preventol 80 Bayer, Germany)	+,1.2 cm	+,1.8 cm	+,1.2 cm	+,1.6 cm	+,2.2 cm	+/–
2	0.89% cremogen henna neutral*	+,0.3 cm	+/–,0.3 cm	—	+,0.5 cm	+,1.3 cm	—
3	0.85% preventol 80 + 0.85% cremogen henna neutral*	+,2.0 cm	+,3.0 cm	+,2.0 cm	+,2.2 cm	+,2.6 cm	+,0.9 cm
4	1% pine oil	—	—	—	—	—	—
5	1% orange terpenes	—	+/–,0.3 cm	—	+/–0.3 cm	+/–	—
6	0.3% IRGASAN	—	+,1.6 cm	+,2.0 cm	+,1.3 cm	+,1.5 cm	—
7	1% black henna AMI**	+,0.3 cm	+/–,0.4 cm	+,0.4 cm	+,0.5 cm	+,0.4 cm	—
8	1% preventol 80 + 1% black henna* AMI**	+,2.4 cm	+,2.6 cm	+,2.6 cm	+,2.4 cm	+,3.2 cm	+,0.6 cm
9	1% preventol 80 + 1% black henna AMI** + 1% orange terpenes	+,2.4 cm	+,2.6 cm	+,2.2 cm	+/–,2.2 cm	+,4.0 cm	+,0.9 cm
10	2% (black henna AMI**)	—	—	+/–	+/–,0.8 cm	+,2.0 cm	+/–
11	1% Bio-Botanica-FE, Neutral henna	—	—	+/–	+,0.5 cm	+,1.0 cm	+/–
12	0.75% Vege-Tech Henna, neutral U.S.A.	+,0.7 cm	+,0.5 cm	++/–,0.3 cm	+,0.8 cm	+,0.7 cm	+,0.5 cm
13	0.85% preventol 80 + 0.85% Vege-Tech Henna, neutral U.S.A.	+,1.3 cm	+,2.2 cm	+,1.8 cm	+,2.6 cm	+,2.8 cm	+,1 cm

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EXAMPLE 2

A group of ten individuals performed the following experiment. Prior to evening shower, the individuals rubbed their right armpits with formulation Pre-shower 3, and their left armpits with placebo (compositions given below). After waiting for three minutes the subjects showered as usual. Microbial counts were estimated directly before application, and the following morning. The individuals themselves, as well as independent judges scored the armpit odors and recorded them, before and after treatment.

Compositions	wt %
1. Pre-shower 3	
Isopropyl myristate	90
Preventol 80	1
Ethanol	8
Perfume (Italy)	1
2. Placebo	
Water	90.5
TAGAT	1
Perfume	0.5
Ethanol	8

The results showed a reduction of 1.5-2 orders of magnitude in bacterial counts in the experimental (right armpit), as compared to no reduction in the control (left armpit).

Similar reductions were observed by the participants in scoring the odor from the armpits, i.e., that the experimental armpit was free or almost free of odor the morning following application, whereas the control armpit had substantial odor. The results show that this invention is highly effective in long-lasting (ca. 8 hours) reduction of microbial counts and odor levels.

EXAMPLE 3

Operating essentially as in Example 2, groups of volunteers tested the following solutions and emulsions:

	wt %
Composition A	
Isopropyl myristate	99
Perfume	1
Composition B	
Isopropylstearate	80
Olive oil	20

-continued

	wt %
Composition C	
Isopropyl palmitate	70
Paraffin oil	27
Tween 80	2
Perfume	1
Composition D	
Octyl palmitate	90
Ethyl alcohol	5
Isopropanol	3
Benzalkonium chloride	2
Composition E	
Isopropyl myristate	95
Silica (Aerosil 200)	3
Benzalkonium chloride	1
Perfume	1
Composition F	
Isopropyl stearate	93
Stearalkonium hectorite	5
Benzalkonium chloride	1
Perfume	1
Composition G	
Mineral oil	90
Glyceryl behenate	8
Cetylpyridinium chloride	1
Perfume	1
Composition H	
Isopropyl stearate	20
Stearyl alcohol	10
Ceteareth-30	3
Benzalkonium chloride	1
Water	65
Perfume	1
Composition I	
Isopropyl myristate	15
Ceteareth-20	5
Cetyl alcohol	8
Benzalkonium chloride	1
Perfume	1
Water	70
Composition J	
Isopropyl stearate	80
sesame oil	18
cetrimonium chloride	1
cetylpyridinium chloride	0.8
Perfume	0.2
Composition K	
White mineral oil	80
n-octanol	10
ethyl alcohol	6
aq. henna extract	3
cetylpyridinium chloride	0.8
Perfume	0.2
Composition L (Cream)	
Isopropyl myristate	83.5
Compritol 888 ATO	15
Preventol R80	1
Perfume	0.5
Composition M (Gel)	
Isopropyl myristate	90.5
Preventol 80	1
Perfume	0.5
Aerosil A200	8
Composition N (Conditioner)	
Isopropyl myristate	15/20
Lanette O	10

-continued

	wt %
Emulgin B2	5
Preventol 80	1
Perfume	0.5
Water	68.5/63.5

The results showed that all of these formulations were effective in reducing axillary odor and bacterial levels.

EXAMPLE 4

Each of the subjects in a group of 75 volunteers were randomly assigned to one of two groups. In one group, the subjects applied Pre-shower 3 of Example 2 to the right armpit, and placebo to the left armpit; the second group vice versa. The compositions of these formulations are given in Example 2 above.

After application of the above formulations, the following assessments were made:

a. axillary odor was scored by a panel of three odor judges;

b. axillary odor was scored by the subjects themselves; and

c. axillae were sampled for bacterial counts.

The statistical significance of the results was assessed by application of Student's paired t-test. Baseline measurements were taken late in the afternoon on day 1. The same night, subjects applied the samples to their axillae and then showered in the normal way, followed by self-assessment of axillary odor. In the morning of day 2, the self-assessment of axillary odor was repeated, and samples for bacterial counts taken. Following this, the subjects showered and, again, performed self-assessment of odor. Late in the afternoon of day 2, subjects were scored for axillary odor by the panel of odor judges, self-assessment was repeated, and axillary bacterial levels sampled.

The results show that according to all three assessment methods, the pre-shower treatment was highly effective ($p<0.001$) as compared with placebo.

All the above description and examples have been given for the purpose of illustration and are not intended to limit the invention in any way. Many modifications can be made in the compositions of the invention. For instance, different oily phases or combinations of two or more such oily phases can be used, many different additives can be incorporated in the compositions of the invention, be they antibacterially active or not, and many different aqueous or organic non-oleous phases can be added, to provide solutions, lotions, emulsions, gels, etc., all without exceeding the scope of the invention.

The invention claimed is:

1. A method of removing body odors, the method comprising the steps of:

(a) priming the skin by applying a deodorizing composition to the axilla region of a human, the deodorizing composition being in the form of an emulsion, the deodorizing composition comprising a first component including an oil selected from the group consisting of vegetable oils, animal oils, synthetic oils, silicone based oils, isopropyl esters, hydrocarbons, and mixtures thereof, wherein the synthetic oils consist of carbon, hydrogen, and oxygen, and a second component including at least one of an antibacterial material and an anti-odor material for a time period of from about 5 seconds to about 5 minutes; and

(b) removing the deodorizing composition after the time period with a detergent.

2. A method according to claim 1, wherein the emulsion comprises water.

3. A method according to claim 1, wherein the antibacterial material comprises benzalkonium chloride.

4. A method according to claim 3, wherein the benzalkonium chloride is at concentrations of about 0.1% (w/v) or higher.

5. A method according to claim 4, wherein the benzalkonium chloride is at a concentration in the range of about 0.1% to 4.0% (w/v).

6. A method according to claim 1, wherein the step of priming the skin comprises applying a deodorizing composition comprising an oil selected from the group consisting essentially of sweet-almond oil, groundnut oil, wheatgerm oil, linseed oil, jojoba oil, apricot stone oil, walnut oil, palm oil, pistachio nut oil, sesame oil, rapeseed oil, cade oil, maize germ oil, peach stone oil, poppy seed oil, pine oil, castor oil, soya oil, avocado oil, safflower oil, coconut oil, hazelnut oil, olive oil, grapeseed oil, sunflower seed oil, whale oil, lard oil, horsehoof oil, tuna oil, caballine oil, otter oil, egg oil, sheep oil, seal oil, turtle oil, halibut liver oil, marmot oil, cod liver oil, neat's-foot oil, and carbon oil.

7. A method of removing body odors, the method comprising the steps of:

(a) priming the skin by applying a deodorizing composition to the axilla region of a human for a time period of from about 5 seconds to about 5 minutes, the deodorizing composition comprising a first component including an isopropyl ester, and a second component including at least one of an antibacterial material and an antiodor material; and

(b) removing the deodorizing composition after the time period with a detergent.

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